Cellular Mechanisms of Renal Adaptation of Sodium Dependent Sulfate Cotransport to Altered Dietary Sulfate in Rats¹

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ABSTRACT

The renal transport and fractional reabsorption of inorganic sulfate is altered under conditions of sulfate deficiency or excess. The objective of this study was to examine the cellular mechanisms of adaptation of renal sodium/sulfate cotransport after varying dietary intakes of a sulfur containing amino acid, methionine. Female Lewis rats were divided into four groups and fed diets containing various concentrations of methionine (0, 0.3, 0.82 and 2.46%) for 8 days. Urinary excretion rates and renal clearance of sulfate were significantly decreased in the animals fed a 0% methionine diet or a 0.3% methionine diet, and significantly increased in the animals fed a 2.46% methionine diet when evaluated on days 4 and 7. Serum sulfate concentrations were unchanged by diet treatment in all animals. The fractional reabsorption of sulfate was significantly increased in the animals fed the 0% methionine diet and the 0.3% methionine diets, and decreased in the animals fed the 2.46% methionine diet. Increased mRNA and protein levels for the sodium/sulfate transporter (NaSi-1) were found in the kidney cortex following treatment with the 0 and 0.3% methionine diet groups. Sulfate homeostasis by renal reabsorption is maintained by an up-regulation of steady state levels of NaSi-1 mRNA and protein when the diet is low in methionine.

Inorganic sulfate is an important physiological anion necessary for the metabolism of many endogenous and exogenous compounds. Xenobiotics including steroids, antiinflammatory agents, analgesics, adrenergic stimulants and blockers undergo biotransformation via sulfate conjugation (Mulder, 1981b). Sulfate conjugation is essential for the biological activity of many endogenous compounds; the degree of sulfation determines the biological activity of heparin, heparan sulfate, dermatan sulfate, gastrin and cholecystokinin (Humphries et al., 1986). Inorganic sulfate is also necessary for biosynthesis of numerous structural components of membranes and tissues, such as glycosaminoglycans or cerebroside sulfate (Humphries et al., 1986).

Inorganic sulfate can be absorbed from the diet or formed from the oxidation of the sulfur containing amino acids, cysteine and methionine. Methionine is rapidly converted to cysteine through the cystathionine pathway, which is the major route for methionine catabolism in the normal adult human (Finkelstein, 1970; Laster et al., 1965). The enzymes involved in this pathway are present ubiquitously in tissues. Although the relative activities of the enzymes vary from tissue to tissue, the liver contains significant quantities of all the enzymes involved in the pathway (Finkelstein, 1970). Cysteine can be formed from methionine and glutathione catabolism, and it is oxidized to inorganic sulfate through the sulfoxidation pathway. Sulfite oxidase readily oxidizes sulfite, the penultimate product of the oxidation pathway of cysteine to sulfate. Inorganic sulfate can also be obtained through the catabolism of sulfated macromolecules, such as glycosaminoglycans, under conditions of fasting or malnutrition (Mulder, 1981a).

Inorganic sulfate is eliminated from the body mainly in unchanged form by urinary excretion (Walser et al., 1953). Normally, renal sulfate clearance is approximately 10 to 35% of the GFR, but approaches GFR when serum sulfate concentrations are increased suggesting saturable reabsorption and negligible, if any, tubular secretion in rat and humans (Becker et al., 1960; Berglund, 1960). Renal reabsorption of sulfate takes place predominantly in the kidney proximal...
tubule. Inorganic sulfate enters into the proximal tubule cell across the BBM via sodium-dependent sulfate cotransport that is distinct from sodium-dependent amino acid, phosphate or glucose cotransport (Lücke et al., 1979; Turner, 1984). Sulfate exits from the proximal tubular cell across the BLM through sulfate anion exchange transport for which bicarbonate is the most effective counterion (Löw et al., 1984; Pritchard and Renfro, 1983).

Several studies have demonstrated adaptive alterations of renal sulfate transport. Sabry et al. (1965) demonstrated a linear relationship between sulfur containing amino acid intake and urinary excretion of sulfate in man. Guinea pigs fed a high sulfate diet exhibited a lower fractional sulfate reabsorption than that in animals fed a low sulfate diet, and animals fed a high sulfate diet manifested marked sulfaturia in response to a sulfate infusion (Neiberger, 1991). Reduced urinary excretion of sulfate was found in rats fed low methionine diets (Rozman et al., 1992). Fernandes et al. (1997) reported adaptive changes in NaSi-1 transport in vitamin D-deficient rats. A decrease in renal BBM NaSi-1 activity was observed associated with a parallel decrease in NaSi-1 protein and mRNA content in vitamin D-deficient rats. Previously, we examined adaptive alterations in renal sulfate transport systems in membrane vesicles, as well as in in vivo renal clearance of sulfate, after the administration of a low methionine diet (Benincosa et al., 1995). The rats fed a low methionine diet had a decreased urinary excretion of sulfate whereas serum sulfate concentrations remained unchanged compared with animals fed a control diet. \( V_{\text{max}} \) for the sodium-dependent sulfate cotransport was increased in BBM isolated from the animals fed a low methionine diet (Benincosa et al., 1995). The results suggested that the renal conservation of sulfate was sufficient to prevent alterations in serum sulfate concentrations, and the sodium-dependent sulfate cotransport was responsible for the adaptive alteration in the renal reabsorption of sulfate.

The cellular mechanism of the adaptation of sulfate renal transport after dietary-induced alterations of inorganic sulfate has not been studied. The cDNA for the sodium-dependent sulfate cotransport (NaSi-1) has been identified by expression cloning and isolated from rat kidney cortex (Markovich et al., 1993). Using the NaSi-1 cDNA, we recently developed assay methods to quantitate mRNA and protein levels for the NaSi-1 transport in tissues. The objective of this study was to investigate renal sulfate reabsorption and cellular mechanisms of the adaptation of the NaSi-1 transport after administration of low and high methionine diets.

**Materials and Methods**

**Dietary treatment.** The sulfur-free diet (a diet without cysteine, methionine and inorganic sulfate) (Table 1) was purchased from Teklad (Madison, WI). Four different diets were prepared by adding various amounts of L-methionine, L-cysteine or inorganic sulfate (Sigma Chemical Co., St. Louis, MO). These include; 1) a diet without cysteine, methionine and inorganic sulfate (0% methionine diet), 2) a diet without cysteine and inorganic sulfate but with 0.3% methionine (0.3% methionine diet), 3) a diet containing 0.82% methionine 0.34% cysteine and 0.12% inorganic sulfate (0.82% methionine; control diet), 4) a diet without cysteine and inorganic sulfate but with 2.46% methionine (2.46% methionine diet). Inorganic sulfate was added to the control diet as potassium sulfate and sodium sulfate in a ratio of 50:50. The concentrations of potassium and sodium were increased 13.5 and 27.5%, respectively when 0.12% sulfate was added to the control diet.

**Study design.** Female Lewis rats (Charles River, Wilmington, MA) weighing 180 to 200 g were randomly divided into four groups and kept in individual cages. The rats received regular laboratory chow (Ralston Purina Co., St. Louis, MO) for 3 days, and then were fed the control synthetic test diet for an additional 3 days. Thereafter, the rats received the test diets and deionized drinking water for 8 days. Food and water were provided to the rats *ad libitum*. Urine was collected for 12 hr on the day before beginning the diets (day 0) and on days 4 and 7. During the urine collection, animals were kept in individual metabolic cages from 7:00 A.M. to 7:00 P.M., and received their drinking water. The diets were restricted during this period to avoid contamination of urine samples with sulfate ion from the diets. A blood sample was obtained at the midpoint of the urine collection period from the tail artery on days 0, 4 and 7. Animals were weighed daily. Animals were sacrificed by CO2 inhalation on day 8 of the study and the kidneys were removed. Kidney cortex was trimmed and immediately frozen in liquid nitrogen. Gastrocnemius muscle was also removed from both right and left legs of the animals, and weighed.

**Sulfate and creatinine analysis.** Serum and urinary sulfate concentrations were measured by single column anion chromatography (Morris and Levy, 1988) with a conductivity detector (Waters 431, Millipore Co., Milford, MA) and an anion-exchange precolumn and analytical column (Wescan Instruments, Santa Clara, CA). A mobile phase of 4 mM potassium hydrogen phthalate, pH 4.2 at a flow rate of 1.6 ml/min was used. The internal standard was potassium iodide. Serum and urinary creatinine concentrations were measured by an alkaline picrate assay described by Darling and Morris (1991).

**TABLE 1**

Composition of the sulfur free diet

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Concentration (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>l-Alanine</td>
<td>3.5</td>
</tr>
<tr>
<td>l-Arginine HCl</td>
<td>12.1</td>
</tr>
<tr>
<td>l-Asparagine</td>
<td>6.0</td>
</tr>
<tr>
<td>l-Aspartic Acid</td>
<td>3.5</td>
</tr>
<tr>
<td>l-Glutamic Acid</td>
<td>40.0</td>
</tr>
<tr>
<td>Glycine</td>
<td>23.3</td>
</tr>
<tr>
<td>l-Histidine HCl · H₂O</td>
<td>4.5</td>
</tr>
<tr>
<td>l-Isoleucine</td>
<td>8.2</td>
</tr>
<tr>
<td>l-Leucine</td>
<td>11.1</td>
</tr>
<tr>
<td>l-Lysine HCl</td>
<td>18.0</td>
</tr>
<tr>
<td>l-Phenylalanine</td>
<td>7.5</td>
</tr>
<tr>
<td>l-Proline</td>
<td>3.5</td>
</tr>
<tr>
<td>l-Serine</td>
<td>3.5</td>
</tr>
<tr>
<td>l-Threonine</td>
<td>8.2</td>
</tr>
<tr>
<td>l-Tryptophan</td>
<td>1.8</td>
</tr>
<tr>
<td>l-Tyrosine</td>
<td>5.0</td>
</tr>
<tr>
<td>l-Valine</td>
<td>8.2</td>
</tr>
</tbody>
</table>

Sucrose  540.797
Corn starch  150.0
Cellulose  50.0
Corn oil  50.0
Vitamin Mix, Teklad (40060)  10.0
Calcium phosphate, dibasic  17.5
Sodium chloride  2.59
Potassium citrate, monohydrate  9.9629
Magnesium oxide  0.84
Magnesium carbonate  0.1225
Ferric citrate  0.21
Zinc carbonate  0.056
Cupric carbonate  0.0105
Potassium iodate  0.0004
Sodium selenite  0.0004
Chromium chloride  0.0103
Tissue RNA preparation. Total RNA was prepared from rat kidney cortex by the guanidium isothiocyanate method (Chirgwin et al., 1979). The tissues isolated from the animals in the same group were combined and ground under liquid nitrogen before the initial homogenization step. Final RNA concentrations in samples were determined by measuring the optical density at 260 nm.

RT-PCR. The primers were derived from the published NaSi-1 cDNA (Markovich et al., 1993) and were designed to produce a 700-bp fragment (native DNA). The 5’ primer was constructed with a BamH-I enzyme digestion site and a portion of the NaSi-1 cDNA corresponding to positions 492–512: CGTGGATCCACCAAGTGTCTGAAAGCAGAAAGCC. The 3’ primer was constructed with a Pst-I enzyme digestion site and a portion of the cDNA corresponding to positions 1172–1192: TCCTTCTGGGCTAACTTAAGGCCAACCTGCTGAGAA. A deletion standard cDNA (600 bp) was prepared by deleting 100 bp of native cDNA located in the middle of the sequence. As a result, the primers used to amplify the native DNA and deletion standard were identical. The cRNA in vitro transcribed from the deletion cDNA was added as an external standard to RT-PCR mixture, and coamplified with sample RNA in the same reaction tube to correct for amplification efficiency.

For the reverse transcriptase reaction, the reagents containing 10 ng tissue RNA, 200 fg deletion standard RNA, 5 mM dithiothreitol, 0.5 μM primers, and 0.1 mM deoxynucleotide triphosphate (dNTP) were mixed in the first strand buffer (50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl2) and first denatured at 75°C for 5 min before 2 U of RNase inhibitor (RNasin, Promega, Madison, WI) and 10 U of SuperScript (Promega) were added. The total reaction volume was 20 μl and the reaction was carried out at 42°C for 45 min. After the reverse transcriptase reaction, additional reagents for polymerase chain reaction (PCR) (10 mM Tris (pH 9.3), 0.4 μM primers, 40 nM dNTP and 3 U/100 μl UltraTaq polymerase) were directly added to the same tubes. After first heating at 95°C for 1 min, 25 cycles were run as follows; 95°C for 1 min, 65°C for 1 min and 72°C for 1 min. Final extension was at 72°C for 7 min and samples were kept at 4°C.

Southern hybridization. The RT-PCR products were size separated on 1.5% agarose gel in TAE (Tris-acetate/ethylenediame tetraacetic acid (EDTA)) buffer and transferred to hybridization matrices (Duralon-UV, Stratagene, La Jolla, CA) using a positive pressure transfer apparatus (Posiblot Transfer Apparatus, Stratagene). The hybridization probe was chosen to contain the sequence present in both native and external standard. The probe (300-bp NaSi-1 cDNA fragment; 492–792 bp) was prepared and amplified by PCR. The random primer labeling reaction was performed using a random primer labeling kit (Prime-It, Stratagene). Matrices were prehybridized for a minimum of 4 hr and hybridized overnight in random primer labeling kit (Prime-It, Stratagene). Matrices were washed five times in 2× SSC, 0.1% SDS at room temperature, then two times in 0.1× SSC, 0.1% SDS at room temperature followed by 0.1× SSC, 0.1% SDS at 65°C until the radioactivity was decreased to background levels. Hybridization signals were visualized and quantitated using a PhosphoImager (Molecular Dynamics, Sunnyvale, CA). The RT-PCR results were expressed as a ratio between amplified NaSi-1 mRNA and amplified deletion standard cRNA, added as an external standard, normalized by the amount of total RNA.

Crude membrane preparation for ELISA. Crude membrane fractions were prepared from kidney cortex to determine the protein expression levels in the tissue. Animals were sacrificed and tissue was harvested and stored in the same manner as described for the total RNA preparations. Approximately 0.25 g of tissue powder was homogenized in the homogenizing buffer (250 mM sucrose, 10 mM triethanolamine-HCl, pH 7.6) and centrifuged at 1250 × g for 10 min at 4°C. The supernatant was further centrifuged at 100,000 × g for 30 min at 4°C (Thomas and McNamee, 1990). The pellet containing crude membrane fractions was resuspended in 2.5% Triton-X in 1 × PBS (sample buffer) to gently extract proteins. Protein concentrations were measured by the method of Lowry et al. (1951). All samples were frozen in liquid nitrogen and stored at −80°C until assayed.

Sandwich-type ELISA procedure. The NaSi-1 polyclonal and monoclonal antibodies were raised against rabbits and mice, respectively (Sagawa et al., 1997). The antigen used for antibody production consisted of a recombinant protein containing 119 amino acids that corresponded to amino acids 159 to 277 of the NaSi-1 protein. The specificity of the raised antibodies was examined by Western analysis using BBM and BLM purified from rat kidney cortex. Both NaSi-1 polyclonal and monoclonal antibodies detected a 69-kDa protein in BBM. The size of the band agreed with a previous report (Lötscher et al., 1996a). The size and the location of our immunoblot suggested that our antibodies recognized NaSi-1 transporter. The NaSi-1 antibody was detected by sandwich-type ELISA (Sagawa et al., 1998). The assay plates (polystyrene flat-bottom microtiter plates, Maxisorp, Nunc, Denmark) were coated with the NaSi-1 monoclonal antibody (10 μg/ml), then incubated with 5% Blotto/PBS overnight at 4°C to block nonspecific absorption. Wells were washed and incubated with samples or sample buffer only (negative control) at 4°C overnight. The wells were incubated with NaSi-1 antisera or pre-immune serum (1:600 diluted in 0.3% BSA/PBS), then incubated with horse radish peroxidase conjugated mouse anti-rabbit IgG (Sigma Immunochemicals, St. Louis, MO). After washing, freshly prepared substrate solution (0.5 mg/ml o-phenylenediamine dihydrochloride, 0.045% H2O2) was added. The reaction was stopped with 2 M sulfuric acid and the OD at 490 nm was measured using a Microkinetics Reader (Bio-Tek instruments, Winooski, VT). The amounts of NaSi-1 protein in the tissue were calculated using a standard curve obtained by a serial dilution of the NaSi-1 standard protein (6.65–164 fmol).

Data analysis. Renal sulfate and creatinine clearances were calculated as the urinary excretion rate divided by the midpoint serum concentration. The sulfate filtration rate was determined from the product of the serum sulfate concentrations and GFR (creatinine clearance) because the serum protein binding of sulfate is negligible. The amount of sulfate reabsorbed was calculated as the amount of sulfate excreted in urine subtracted from the total amount filtered. The fraction of the filtered sulfate which was reabsorbed was calculated by 1-C/GFGR.

Statistical analysis. All results are expressed as the mean ± S.D. The data obtained after each diet were compared to day 0 values with a paired t-test. The values between the groups were compared using one-way analysis of variance. Posttests were performed with the Dunnett multiple comparisons test.

Results

Weight gain. Rats that received the 0.3% methionine diet and the 2.46% methionine diet gained weight at the same rate as rats fed the control diet (fig. 1). However, rats maintained on the 0% methionine diet had significantly decreased body weights beginning on day 2 of the diets (P < 0.01, n = 6). Gastrocnemius muscle obtained from rats that received the 0% methionine diet weighed significantly less than that obtained from rats that received the control diet (P < .05, fig. 2). When the muscle weight was normalized with the body weight, there were no differences among groups, which indicated that the body weight loss was due, to a large degree, to the loss of muscle mass.

Renal reabsorption of sulfate and serum sulfate concentration. The mean values of serum sulfate concentration, urinary excretion rate, renal sulfate clearance and renal reabsorption of sulfate on days 0, 4 and 7 in each study group are shown in table 2. There were no significant differences in all the parameters measured on day 0 among the groups.
There were no significant differences in serum sulfate concentrations in each study group on days 4 or 7 compared with the values obtained on day 0. Urinary excretion of sulfate was significantly decreased in the animals fed the 0% methionine diet (P < .001, n = 6) and 0.3% methionine diet (P < .05, n = 5), and increased in the animals fed the 2.46% methionine diet on days 4 and 7 compared to the value for day 0 (P < .01, n = 6). The renal sulfate clearance values in the 0% methionine diet group and 0.3% methionine group approached zero after 4 days of the diet, and the changes on days 4 and 7 were statistically significant compared with the value on day 0. The renal sulfate clearance values were increased significantly after 4 and 7 days of the 2.46% methionine diet compared to the values for day 0 (P < .05, n = 5). The fraction of sulfate reabsorbed was significantly increased and was 97 to 99% in the 0 and 0.3% methionine groups after 4 days of the diets. The animals that received the 2.46% methionine had a significantly decreased fraction of sulfate reabsorbed compared to the values on day 0 (P < .05, n = 6). There were no significant differences in creatinine clearance after treatment with any of the diets.

**NaSi-1 mRNA.** Kidney cortex obtained from the animals in each diet group were pooled. Total RNA was isolated in quadruplicate from each tissue pool, and RT-PCR was performed in duplicate for each RNA preparation. The hybridization signals are shown in figure 3A. The results demonstrated that the steady state NaSi-1 mRNA level was significantly increased in animals that received the 0% methionine diet (P < .01, n = 8) and the 0.3% methionine diet (P < .05, n = 8) compared to the control group after 8 days of treatment (fig. 3B). The mean mRNA value was decreased by 19.4% in animals fed the 2.46% methionine diet but the value was not statistically different from the mean control value.

**NaSi-1 protein abundance.** A crude membrane fraction was isolated from the rat kidney cortex obtained from animals after 8 days of the various diets. ELISA was performed in triplicate from duplicate crude membrane preparations from each study group. The NaSi-1 protein abundance was significantly higher in the kidney cortex obtained from the animals that received the 0% methionine diet (P < .01, n = 6), and the 0.3% methionine diet (P < .05, n = 6) than in the control group. There was no significant difference in NaSi-1 protein abundance between the 2.46% methionine and the control groups although the mean value in the 2.46% methionine treated group was 20% lower than the mean value in the control group (fig. 4).

**Previous diet study.** We previously reported significantly decreased sulfate urinary excretion in vivo and decreased sodium/sulfate transport in isolated membrane vesicles isolated from the kidney cortex from rats fed a low methionine diet (0.37% methionine) compared with a control diet (1.12% methionine) (Benincosa et al., 1995). The diets used in our previous study (Benincosa et al., 1995) were obtained from ICN Pharmaceuticals (Aurora, OH) and differ from those used in the current study. The 0.37% methionine in the low methionine diet was the sole source of sulfur. The control diet contained 1.12% methionine as well as 0.07% cysteine and the sulfate salt form of various electrolytes. We repeated the studies with these diets and measured the NaSi-1 mRNA and protein abundance in kidney cortex after the treatment for 8 days. The NaSi-1 mRNA and protein levels were significantly higher in the animals fed the low methionine diet compared with the control diet (fig. 5).

**Discussion**

Our in vivo data are consistent with previous reports; sulfate deprivation induced by a diet low in methionine results in a significant decrease in the urinary excretion rate and renal clearance of sulfate, although the serum sulfate concentrations are unchanged (Benincosa et al., 1995; Rozman et al., 1992). The animals fed the 0% methionine diet exhibited growth retardation due to the loss of muscle mass. Because the serum sulfate concentrations in the animals fed a 0% methionine diet were similar to those in control
animals, it appears that these animals maintained normal serum sulfate levels through the catabolism of muscle. Urinary excretion rate and renal clearance of sulfate were decreased in the animals fed the 0% methionine diet and the 0.3% methionine diet. The animals fed the 0.3% methionine diet did not lose body weight and muscle weight and the serum sulfate concentrations were unchanged, which suggested that the adaptive changes in renal sulfate reabsorption were sufficient to maintain sulfate homeostasis without muscle catabolism in this diet group.

In the 0% methionine and the 0.3% methionine groups, the fraction of sulfate reabsorbed was significantly increased (approximately 25% increase), and the values approached 100% which indicated that the amount of sulfate filtered by the kidney was completely reabsorbed in renal proximal tubules. This result agrees with our results showing that both the urinary excretion and the renal clearance of sulfate approached zero. The NaSi-1 transport protein level was increased by 73 and 31% in the 0 and 0.3% methionine groups, respectively.

### Table 2

Effect of diet on sulfate serum concentration, urinary excretion, renal clearance and renal reabsorption

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0% Meth.</th>
<th>0.3% Meth.</th>
<th>0.82% Meth.</th>
<th>2.46% Meth.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum concentration (mM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>0.62 ± 0.23</td>
<td>0.60 ± 0.13</td>
<td>0.63 ± 0.12</td>
<td>0.62 ± 0.13</td>
</tr>
<tr>
<td>Day 4</td>
<td>0.37 ± 0.21</td>
<td>0.62 ± 0.14</td>
<td>0.56 ± 0.07</td>
<td>0.86 ± 0.35</td>
</tr>
<tr>
<td>Day 7</td>
<td>0.54 ± 0.36</td>
<td>0.67 ± 0.20</td>
<td>0.73 ± 0.15</td>
<td>0.89 ± 0.35</td>
</tr>
<tr>
<td>Urinary excretion rate (µmol/12 hr/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>341 ± 164</td>
<td>308 ± 216</td>
<td>331 ± 243</td>
<td>347 ± 81.2</td>
</tr>
<tr>
<td>Day 4</td>
<td>28.7 ± 42.0**</td>
<td>20.6 ± 23.1*</td>
<td>290 ± 156</td>
<td>1170 ± 235***</td>
</tr>
<tr>
<td>Day 7</td>
<td>12.3 ± 16.6**</td>
<td>32.5 ± 24.1*</td>
<td>435 ± 253</td>
<td>1690 ± 282***</td>
</tr>
<tr>
<td>Renal clearance (ml/min/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>0.81 ± 0.41</td>
<td>0.51 ± 0.40</td>
<td>0.73 ± 0.50</td>
<td>0.82 ± 0.36</td>
</tr>
<tr>
<td>Day 4</td>
<td>0.08 ± 0.09**</td>
<td>0.04 ± 0.04*</td>
<td>0.69 ± 0.45</td>
<td>2.03 ± 0.80*</td>
</tr>
<tr>
<td>Day 7</td>
<td>0.02 ± 0.02**</td>
<td>0.07 ± 0.06*</td>
<td>0.75 ± 0.29</td>
<td>2.98 ± 1.15*</td>
</tr>
<tr>
<td>Fractional reabsorption (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>78.1 ± 9.20</td>
<td>83.3 ± 9.88</td>
<td>75.8 ± 1.20</td>
<td>76.5 ± 4.1</td>
</tr>
<tr>
<td>Day 4</td>
<td>97.4 ± 2.79**</td>
<td>98.6 ± 1.40*</td>
<td>73.9 ± 6.41</td>
<td>58.6 ± 10.1*</td>
</tr>
<tr>
<td>Day 7</td>
<td>99.2 ± 1.00**</td>
<td>96.7 ± 3.94*</td>
<td>71.1 ± 6.03</td>
<td>43.4 ± 27.4*</td>
</tr>
</tbody>
</table>

![Fig. 3.](image)

**Fig. 3.** A, Southern hybridization signals of RT-PCR products. The top bands are reverse transcribed and amplified NaSi-1 RNA in total RNA prepared from rat kidney cortex. Lanes are duplicates of RNA preparations for each diet group. The bottom bands are reverse transcribed and amplified deletion RNA added as an external standard. B, The results of RT-PCR for the NaSi-1 mRNA in the tissue. The values are expressed as a ratio of NaSi-1 mRNA to external standard RNA normalized with amounts of total RNA used for RT-PCR reactions. The data are mean ± S.D. of duplicate lanes of two RNA preparations of pooled kidney cortex from each diet group. * P < .05, ** P < .01, significantly different from the control group.

![Fig. 4.](image)

**Fig. 4.** NaSi-1 transport protein levels in the kidney cortex after 8 days of various sulfate containing diets. The values are mean ± S.D. of triplicate measurements from duplicate membrane preparations in a pooled sample from each study group. * P < .05, ** P < .01, significantly different from the control group.

0.3% methionine diet. The animals fed the 0.3% methionine diet did not lose body weight and muscle weight and the serum sulfate concentrations were unchanged, which suggested that the adaptive changes in renal sulfate reabsorption were sufficient to maintain sulfate homeostasis without muscle catabolism in this diet group.

In the 0% methionine and the 0.3% methionine groups, the fraction of sulfate reabsorbed was significantly increased (approximately 25% increase), and the values approached 100% which indicated that the amount of sulfate filtered by the kidney was completely reabsorbed in renal proximal tubules. This result agrees with our results showing that both the urinary excretion and the renal clearance of sulfate approached zero. The NaSi-1 transport protein level was increased by 73 and 31% in the 0 and 0.3% methionine groups,
respectively. The NaSi-1 mRNA level was also significantly increased in the 0% methionine and the 0.3% methionine groups. These findings suggest that mRNA level of the NaSi-1 transport protein is increased when methionine is either absent from the diet or present at a low concentration, resulting in an increased amount of transport protein. We confirmed our mRNA and protein abundance results by examining a different low methionine diet that we had used previously in a study in which changes in sodium/sulfate co-transporter (NaPi-2) are involved in the adaptive response to decreased dietary phosphate content (0.1% phosphate) under chronic conditions (7 days) (Lötscher et al., 1996b). The sodium-coupled glucose transporter (SGLT) activity and mRNA levels are increased in the porcine kidney epithelial cell line LLC-PK1, maintained in the presence of a culture medium containing low glucose (5–10 mM) as compared with that containing high glucose (25 mM) (Moran et al., 1983; Ohta et al., 1990).

In the animals fed the high methionine diet, there were no significant changes in the levels of NaSi-1 mRNA or protein, although fractional reabsorption of sulfate was significantly decreased compared to the levels in animals fed the control diet. In this group, urinary excretion and renal clearance of sulfate were significantly increased after 8 days of diet, although the serum sulfate concentrations did not change. Therefore, decreased fractional reabsorption of sulfate without significant changes in mRNA or protein levels of NaSi-1 transport appeared to be due, at least in part, to the increased urinary excretion of sulfate resulting from the saturation of the reabsorption process after ingestion of a diet containing 2.46% methionine. The mean NaSi-1 protein level in this group was decreased by 20% compared to that in control animals, although this change was not statistically significant. The magnitude of the change was similar to that observed in the 0.3% methionine group, but the variation was larger in the 2.46% methionine group. Hence, it is possible that the NaSi-1 protein level is actually decreased in these animals and that represents the mechanism of decreased fractional reabsorption of sulfate. An additional study using a larger number of animals to improve the statistical power will be necessary to address this issue. It is also possible that the increased excretion of sulfate in this study group is due to an increased secretion, in addition to the saturation of the reabsorptive mechanism. Sulfate secretory mechanisms have been postulated in the literature (Brazy and Dennis, 1981).

Our investigation is the first study demonstrating the cellular mechanism of renal adaptation to diets varying in methionine (the precursor of inorganic sulfate). Little is known regarding the regulation of sulfate homeostasis. Inorganic sulfate is essential for sulfate conjugation reactions that are responsible not only for the detoxification of many endogenous and exogenous compounds, but also for the biosynthesis of biologically active compounds. Numerous structural components of membranes and tissues are sulfate conjugates: sulfated glycosaminoglycans are components of cartilage and other tissues (DeMeio, 1975), although cerebroside sulfate is a constituent of the myelin membrane in the brain (Grumet et al., 1993). In tissues, sulfated glycosaminoglycans occur as covalent complexes with a core protein in the form of proteoglycans and cell differentiation appears to be guided by a tissue-specific composition of sulfated proteoglycans (Dietrich et al., 1977). The importance of proteoglycan sulfation has been clearly recognized after the identification of three chondrodysplasias (achondrogenesis type 1B, atelosteogenesis type 2 and diastrophic dysplasia) (Superti-Furga et al., 1996). These diseases result from a deficient sulfate transport and are characterized by deficient intracellular pools of sulfate in chondrocytes, leading to the production of undersulfated proteoglycans and the clinical features of these chondrodysplasias including dwarfism, spinal deformation and joint abnormalities (Hastbacka et al., 1994).
In summary, our study demonstrated that 1) urinary excretion and renal clearance of sulfate were decreased in animals after ingestion of a low methionine diet, and increased following a high methionine diet, 2) the serum sulfate concentrations were constant in all the study groups even in those animals receiving a high methionine diet, 3) the amounts of NaSi-1 mRNA and transport protein were increased after ingestion of a low methionine diet and 4) a diet containing 2.46% methionine (a high methionine diet) did not significantly alter the NaSi-1 mRNA or protein levels although the lack of significance may be due to the variability observed in this study group. From these results, it can be concluded that sulfate homeostasis is maintained by an up-regulation of steady state levels of the renal sulfate transport mRNA and protein when the diet is low in methionine.

References

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