Proteomic Analysis Reveals Changes in the Liver Protein Pattern of Rats Exposed to Dietary Folate Deficiency

Aurélie Chanson,* Thierry Sayd,* Edmond Rock, Christophe Chambon,† Véronique Santé-Lhoutellier,* Geneviève Potier de Courcy,** and Patrick Brachet2

ABSTRACT  Epidemiologic and experimental studies showed that folate deficiency is associated with increased risk of degenerative diseases by enhancing abnormal one-carbon metabolism. We used the changes in the proteome of liver, the main tissue of folate storage and metabolism, in a rat model of dietary folate depletion. Four-month-old rats were fed for 4 wk an amino acid–defined diet without folate and compared with pair-fed rats given the same diet adequately supplemented with folic acid. Folate deprivation decreased plasma and hepatic folate concentrations dramatically, while increasing homocysteinemia significantly. Using 2-dimensional electrophoresis and matrix-assisted laser desorption/ionization time-of-flight MS, we identified 9 spots corresponding to differentially expressed proteins in the liver of folate-deficient rats compared with controls. Among those spots, 4 had a significantly increased volume, whereas the volume of the 5 other spots was decreased. Upregulated proteins included glutathione peroxidase (GPx) 1 and peroxiredoxin 6, 2 enzymes involved in the response to oxidative stress, and MAWDP binding protein (MAWDBP), which has been associated with cancer. MAWDBP was simultaneously identified as a second spot with a lower isoelectric point (pI) that vanished almost completely after folate deficiency. Decreased abundance was also observed for cofilin 1, a protein linked to tumorigenesis, and for the GRP 75 precursor and preproalbumin, both of which are responsive to oxidative stress and/or inflammation. Moreover, an enzyme assay and/or Western blot analysis of GPx-1 and MAWDBP confirmed the proteomic findings. Our results show that folate deficiency modifies the abundance of several liver proteins consistently with adaptive tissue responses to oxidative and degenerative processes.  J. Nutr. 135: 2524–2529, 2005.

KEY WORDS: ● proteomics ● antioxidant enzymes ● homocystein ● hepatic proteins ● folate deprivation

Folate, a generic term for all compounds that exhibit vitamin activity similar to that of pteroylmonoglutamic acid (folic acid), plays a fundamental physiological role in 1-carbon metabolism (1). Folate nutritional status depends on intake from food and folic acid supplements as well as on the bioavailability of the various ingested forms of this vitamin (2). In developed countries, severe folate deficiency is uncommon, but specific population subgroups, e.g., pregnant or lactating women and elderly subjects, may be at risk for moderate folate deficiency (3,4). Folates are currently under intense scrutiny for their ability to modulate disease risk. Periconceptual voluntary supplementation of women or mandatory fortification for their ability to modulate disease risk. Periconceptual voluntary supplementation of women or mandatory fortification

3 Abbreviations used: AKR1C9, aldo-keto-reductase 1C9; C, control; 2-DE, 2-dimensional electrophoresis; FD, folate-depleted; GPx, glutathione peroxidase; GRP 75, glucose-regulated protein 75; Hcy, homocysteine; 3-α-HSD, 3-α-hydroxysteroid dehydrogenase; IEF, isoelectric focusing; I.N., index number; IPG, immobilized pH gradient; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight MS; MAPK, mitogen-activated protein kinase; MAWDBP, putative MAPK activator with WD repeats; pI, isoelectric point; PMF, peptide mass fingerprinting; Prdx, peroxiredoxin; tHcy, total homocysteine; WD, tryptophan aspartic acid.
risk factor for cardiovascular diseases (6,11) and is associated with cognitive decline and neuropsychiatric disorders such as Alzheimer’s disease (12,13). The putative mechanisms of the adverse effects of Hcy on cells include oxido-reduction reactions, activation of proliferation- or apoptosis-signaling pathways, and alteration of gene expression (7,14). Numerous studies have focused on the Hcy-lowering effect of natural folate and its possible protective effects against degenerative diseases (4,6,10,15). Additionally, disruption of DNA integrity through chromosomal breaks and uracil misincorporation, alteration of DNA repair, and/or change in the expression of critical tumor suppressor genes and protooncogenes could increase the risk of cancer in subjects with low folate status (8,16). Nevertheless, more data are required to clearly explain the cellular and molecular mechanisms that underlie metabolism changes associated with folate deficiency.

The present study was designed to improve our understanding of these changes in the liver of rats subjected to dietary folate depletion. Liver constitutes an important tissue for folate metabolism, and decreased folate concentration and disturbed 1-carbon metabolism take place in this tissue during folate deficiency (17,18). However, little is known about the changes that occur concomitantly at the level of abundance of hepatic proteins. These aspects were investigated by a proteomic analysis of the liver of rats fed a diet without folate for 4 wk compared with pair-fed rats given the same diet adequately supplemented with folic acid. Differentially expressed proteins were identified using 2-dimensional electrophoresis (2-DE) and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS. Possible connections of these proteins with degenerative diseases associated with folate deficiency were discussed.

**MATERIALS AND METHODS**

**Diet, rats, and tissue samplings.** Pellets of an L-amino acid-defined diet (19) supplemented with 1.5 or 0 mg folic acid/kg diet, comprising the control (C) or folate-depleted (FD) diet, respectively, were synthesized by the INRA-Unité de Préparation des Aliments Expérimentaux. Both diets contained the antibiotic succinylsulfoximine of 5 mmol/L of sodium ascorbate. All plasma aliquots were stored for folate analysis, plasma aliquots were supplemented with 0.1 vol-

ty phosphine, 0.2% Biolytes], and the homogenate was centrifuged at 10,000 × g for 1 h. The protein concentration of the supernatant was determined using the Bio-Rad RC DC protein assay kit.

**Frozen liver pieces** were homogenized in extraction buffer [5 mol/L of urea, 2 mol/L of thiourea, 4% (v:v) CHAPS, 40 mmol/L of Tris, 2 mmol/L of tributylphosphate, 0.2% Biolytes], and the homogenate was centrifuged at 100,000 × g for 1 h. The protein concentration of the supernatant was determined using the Bio-Rad DC protein assay kit. For immobilized pH gradient (IPG) isoelectric focusing (IEF), 300 or 1000 μg of proteins (for analytical or preparative gels, respectively) were loaded onto 17-cm Bio-Rad ReadyStrips, pH 3–10 nonlinear, by inclusion of an adequate volume of extract in rehydration buffer [9 mol/L of urea, 4% (v:v) CHAPS, 0.1 mol/L of dithiothreitol, 0.2% biolytes, 0.0002% (v:v) Bromophenol blue]. Passive rehydration of the strips was carried out over 13 h. The IEF consisted in increasing the voltage gradually from 250 to 5000 V over a 20.5-h period. After equilibration of the IPG strips, SDS-PAGE was performed on 12% polyacrylamide gels. Separate protein spots were visualized on analytical or preparative gels by silver staining or 0.02% (v:v) colloidal Coomassie blue staining, respectively.

**Image analysis.** Gel images were acquired and analyzed using the PDQuest software (Bio-Rad) (24). For a given gel, the volume of each protein spot was calculated (ppm) by dividing its raw volume by the sum of the volumes of all valid spots. Normalized volumes were calculated for each spot, and the spot volume ratio was calculated for each gel.

**In-gel digestion, desalting, concentration, and MALDI-TOF MS identification of protein spots.** All of these steps were performed essentially as described previously (24). Peptide mass fingerprints (PMF) were compared with mammalian databases (NCBI nonredundant and SWISS-PROT) (25,26). The search criteria used were 1 missing trypsin cleavage site, partial carbamidomethylation of cysteine, partial methionine oxidation, and a mass deviation of ±30 ppm. Z-scores were defined by comparison of search results against estimated random match population. Z-scores > 1.65 were considered significant (P < 0.05). All of the protein identifications were in the expected size range based on position in the gel.

**Western blot assays.** Liver extracts were prepared in 1 mmol/L of EDTA:0.13 mmol/L of BHT:100 mmol/L of phosphate buffer pH 7.4 (0.5 g of liver in 5 mL of buffer) before centrifuging twice at 10,000 × g for 10 min at 4°C. Supernatant proteins (2 μg) were separated by SDS-PAGE on a 15% (v:v) polyacrylamide gel and Western blots were carried out as described previously (27), using rabbit antibody to human glutathione peroxidase 1 (GPx 1; Acris Antibodies) at 1:5000 dilution and the Amersham ECF Western-blotting kit. For the putative mitogen-activated protein kinase (MAPK) activator with tryptophan acidic protein (WDP) repeats (MAWD) binding protein (MAWDDBP) assay, Western blots were performed on liver protein extracts resolved by 2-DE gels, as explained above. On the basis of the proteomic data, an area of ~50 cm² around the MAWDDBP protein spots was cut from the 2-DE gels and processed for Western blot analysis using rabbit antibody to rat MAWDDBP (a kind gift from Dr. A. Pawlak, INSERM U851, Créteil, France) at 1:4000稀释.

**Assay of liver GPx activity.** GPx specific activity (U/mg protein) was determined in liver extracts according to previously published procedures (28,29). Protein concentration of liver extracts was determined with Bradford assay (Bio-Rad) following the supplier’s instructions.

**Folate and homocysteine assays.** Frozen liver pieces were homogenized in ice-cold phosphate extraction buffer [0.1 mol/L of sodium phosphate pH 7, 0.2 mol/L of mercaptotoanol and 2% (v:v) sodium ascorbate as antioxidants] and then placed into a boiling water bath for 10 min to precipitate the proteins. After cooling over ice, the homogenate was stirred and centrifuged at 12,000 × g for 10 min. Then, a supernatant aliquot was incubated at pH 7 for 3 h in a shaking water-bath at 37°C with 0.2 volumes of chicken pancreas conjugase extract (Difico Labs, BD) (10 g/L) to hydrolyze liver folylpolyglutamates.

Plasma and liver folate concentrations were measured by microbiological assay using Lactobacillus casei ATCC 7469 (L. rhamnosus; Institut Pasteur) and free folic acid-case medium (Difico Labs) (22). Plasma Hcy concentrations were determined by HPLC and fluorometric detection (Waters), using the HPLC Reagent Kit from Bio-Rad Laboratories following the supplier’s instructions (22).

**2-DE of liver proteins.** 2-DE was performed as previously described (23,24) with some modifications. Importantly, all 2-DE experiments were carried out simultaneously to optimize the analytical reproducibility. Frozen samples of liver from C or FD rats were homogenized in extraction buffer [5 mol/L of urea, 2 mol/L of thiourea, 4% (v:v) CHAPS, 40 mmol/L of Tris, 2 mmol/L of tributylphosphate, 0.2% Biolytes], and the homogenate was centrifuged at 100,000 × g for 1 h. The protein concentration of the supernatant was determined using the Bio-Rad DC protein assay kit.

**Image analysis.** Gel images were acquired and analyzed using the PDQuest software (Bio-Rad) (24). For a given gel, the volume of each protein spot was calculated (ppm) by dividing its raw volume by the sum of the volumes of all valid spots. Normalized volumes were calculated for each spot, and the spot volume ratio was calculated for each gel.

**In-gel digestion, desalting, concentration, and MALDI-TOF MS identification of protein spots.** All of these steps were performed essentially as described previously (24). Peptide mass fingerprints (PMF) were compared with mammalian databases (NCBI nonredundant and SWISS-PROT) (25,26). The search criteria used were 1 missing trypsin cleavage site, partial carbamidomethylation of cysteine, partial methionine oxidation, and a mass deviation of ±30 ppm. Z-scores were defined by comparison of search results against estimated random match population. Z-scores > 1.65 were considered significant (P < 0.05). All of the protein identifications were in the expected size range based on position in the gel.

**Western blot assays.** Liver extracts were prepared in 1 mmol/L of EDTA:0.13 mmol/L of BHT:100 mmol/L of phosphate buffer pH 7.4 (0.5 g of liver in 5 mL of buffer) before centrifuging twice at 10,000 × g for 10 min at 4°C. Supernatant proteins (2 μg) were separated by SDS-PAGE on a 15% (v:v) polyacrylamide gel and Western blots were carried out as described previously (27), using rabbit antibody to human glutathione peroxidase 1 (GPx 1; Acris Antibodies) at 1:5000 dilution and the Amersham ECF Western-blotting kit.

**Assay of liver GPx activity.** GPx specific activity (U/mg protein) was determined in liver extracts according to previously published procedures (28,29). Protein concentration of liver extracts was determined with Bradford assay (Bio-Rad) following the supplier’s instructions.
Statistics. Results were expressed as means ± SD. Statistical analyses were performed using V3.00 GraphPad InStat (GraphPad Software). The statistical significance of differences between means of FD and C rat groups was assessed using the 2-tailed Student’s t test or Mann-Whitney test, with P < 0.05 considered significant. Correlations were assessed using the Pearson’s correlation coefficient (r).

RESULTS

Biological and biochemical characteristics of folate-depleted adult rats. Compared with the pair-fed controls (C), body weight (515 ± 40 and 507 ± 17 g), liver weight (11.7 ± 1.7 and 12.1 ± 1.5 g), and hematocrit (42.2 ± 2.8 and 39.2 ± 3.0%), of rats subjected to dietary folate depletion for 4 wk (FD) were not affected. On the other hand, folate depletion caused a 91 and 80% decrease in plasma and liver folate concentrations, respectively (Table 1). Folate deficiency was also accompanied by a 3.6-fold increase in the plasma tHcy concentration of the rats. When the individual data of rats of both groups (n = 13) were pooled, plasma folate and tHcy concentrations (r = −0.82, P = 0.0005) and hepatic folate and plasma tHcy concentrations (r = −0.81, P = 0.0007) were negatively correlated (data not illustrated).

Changes in the liver proteome of folate-depleted adult rats. In preliminary experiments, for each group, 2-DE gels were done in triplicate with a mixture of liver protein extracts obtained from 3 different rats. Triplicate gels obtained from a done in triplicate with a mixture of liver protein extracts

The magnitude of the decreases [(C spot volume – FD spot volume) × 100/C spot volume] observed was 40.8, 33.8, 38.9, and 47.4%, respectively.

Confirmation of proteomic data by Western blot analysis and enzyme activity assay. To verify the accuracy of the proteomics results, the amounts of GPx 1 protein in liver homogenates from FD and C rats (n = 4 rats/group) were compared using Western blot analysis with a polyclonal antibody specific to GPx 1 (Fig. 3A). Fluorescence quantification of the immunoreactive protein confirmed that GPx 1 protein abundance was increased 1.43 fold (P = 0.035) in the liver of FD rats [1.88 ± 0.33 arbitrary U (× 10⁶) vs. 1.32 ± 0.25 arbitrary U (× 10⁶) in controls]. Additionally, measurement of enzyme activity in the same homogenates indicated that dietary folate deficiency increased (P = 0.0019) the specific activity of liver GPx from 1.01 ± 0.07 (C rats, n = 4) to 1.41 ± 0.14 U/mg proteins (FD rats, n = 4) (data not illustrated). Finally, probing 2-DE-separated liver homogenates by Western blotting with a rabbit polyclonal antibody specific to human MAWDPB confirmed the identity of the 2 protein spots 4112 and 4117 (Fig. 3B). It also showed that spot 4117 was no longer evident in samples from the liver of FD rats, in accordance with the result of silver-stained 2-DE gels (Fig. 2D).

TABLE 1

Changes in folate status and homocysteinemia after folate depletion in adult rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Folate-depleted</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Plasma folate, nmol/L</td>
<td>159.6 ± 12.7</td>
<td>14.1 ± 1.1*</td>
</tr>
<tr>
<td>Liver folate, nmol/g wet tissue</td>
<td>20.5 ± 2.2</td>
<td>4.0 ± 1.0*</td>
</tr>
<tr>
<td>Plasma homocysteine, μmol/L</td>
<td>6.4 ± 1.8</td>
<td>22.9 ± 8.6*</td>
</tr>
</tbody>
</table>

* Values are means ± SD. * Different from control according to Mann-Whitney test (P < 0.05).

DISCUSSION

Liver is an important tissue in folate metabolism, and long-term folate deficiency was shown to disturb 1-carbon metabolism (14,17,18,31). The aim of the present study was to determine the changes in the liver proteome of adult rats after 4 wk of consuming a folate-free diet. Such a nutritional condition is sufficient to cause a substantial decrease in the folate content of plasma and liver concurrently with an increase in plasma tHcy; for the first time, we showed that this leads to
indicative of a tissue response to an oxidative stress induced by folate depletion. Oxidative stress was observed previously in folate-deficient patients (15,34,35), weaning rats (31,36), and cultured cells (37). However, liver total GPx activity was unchanged in weaning rats fed a FD diet compared with age-paired rats fed a diet containing 2 mg of folic acid/kg (31); the latter nutritional condition is similar to that used here as the control condition. Age-dependent regulation of GPx (38) could explain the different responses of this antioxidant enzyme to the oxidative stress induced by folate deficiency in adult and weaning rats.

In the present study, plasma tHcy concentration rose from 6.4 μmol/L in C rats to 22.9 μmol/L in FD rats concomitantly with an increased abundance of hepatic GPx 1 and Prdx 6. Such a variation in plasma tHcy concentration is in the range of that reported for mild hyperhomocysteinemia in humans (13). Moat et al. (39) observed previously that plasma tHcy concentrations ≥ 20 μmol/L in humans are associated with increased activity of circulating antioxidant enzymes including plasma GPx. This was attributed, at least in part, to an increase in the amount of enzyme protein, perhaps due to a protective reducing effect of thiols on GPx. Moreover, dietary folate deficiency in mice was reported to result in increased glutathione levels in brain tissue (40). Overall, the last-mentioned 2 studies and the present one support the in vivo existence of compensatory mechanisms to counteract the oxidative stress generated by folate deficiency. Also possibly related to the cellular effects of Hcy is the precursor of DNA-type molecular chaperone glucose-regulated protein 75 (GRP75), which is one of the proteins presently identified as having a decreased abundance in the liver of FD rats. GRP75 is also known as mitochondrial heat shock protein 70 (HSP70) (41). In 2 populations with frequently elevated homocysteinemia and/or unbalanced redox status, i.e., older subjects and patients with Alzheimer’s disease, decreased HSP70 expression was observed in olfactory receptor neurons or mononuclear blood cells, respectively (42,43). Whether mild hyperhomocysteinemia or oxidative stress in FD rats may contribute to the decreased abundance of liver GRP75 precursor remains to be determined. Moreover, the in vivo contribution of Hcy-independent mechanisms, e.g., deficiency of supposedly anti-

### TABLE 2

<table>
<thead>
<tr>
<th>Protein index number (I.N.)</th>
<th>Protein name</th>
<th>Control</th>
<th>Folate-depleted</th>
<th>Change in protein abundance</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3103</td>
<td>Prdx 6</td>
<td>9563 ± 1052</td>
<td>11458 ± 1525</td>
<td>↑ 1.20×</td>
<td>0.038</td>
</tr>
<tr>
<td>4112</td>
<td>MAWDBP</td>
<td>3174 ± 534</td>
<td>4921 ± 1351</td>
<td>↑ 1.55×</td>
<td>0.028</td>
</tr>
<tr>
<td>5012</td>
<td>GPx 1</td>
<td>7945 ± 736</td>
<td>10631 ± 2148</td>
<td>↑ 1.34×</td>
<td>0.018</td>
</tr>
<tr>
<td>6230</td>
<td>3-α-HSD</td>
<td>3387 ± 466</td>
<td>5021 ± 1426</td>
<td>↑ 1.48×</td>
<td>0.026</td>
</tr>
<tr>
<td>2323</td>
<td>3(2),5-bisphosphate nucleotidase 1</td>
<td>487 ± 175</td>
<td>268 ± 70</td>
<td>↓ 40.8%</td>
<td>0.042</td>
</tr>
<tr>
<td>2735</td>
<td>DNAK-type molecular chaperone grp 75 precursor</td>
<td>1503 ± 291</td>
<td>994 ± 278</td>
<td>↓ 33.8%</td>
<td>0.016</td>
</tr>
<tr>
<td>3739</td>
<td>Preproalbumin, 608 aa</td>
<td>19890 ± 5508</td>
<td>12159 ± 3542</td>
<td>↓ 38.9%</td>
<td>0.025</td>
</tr>
<tr>
<td>4117</td>
<td>MAWDBP</td>
<td>3562 ± 622</td>
<td>1.2 ± 0.6</td>
<td>↓ 99.9%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>8003</td>
<td>Cofilin 1</td>
<td>2721 ± 858</td>
<td>1431 ± 499</td>
<td>↓ 47.4%</td>
<td>0.016</td>
</tr>
</tbody>
</table>

1 Values are means ± SD (n = 6 and 5 in the control and folate-depleted group, respectively). P values derived from statistical analysis using Student’s t test.
2 Fold increase (with up arrow) or percent of decrease (with down arrow) in the normalized volume of a given protein spot in the liver of folate-depleted rats vs. control rats.

### FIGURE 2

Differentially expressed protein spots in the liver proteome of adult rats fed the FD or C diet. Bold-framing of spots in expanded images indicates significantly higher volumes when comparing the 2 experimental conditions (see Table 2).
Summary of differentially abundant proteins identified by proteomics in rat liver in response to dietary folate depletion

<table>
<thead>
<tr>
<th>Identified protein</th>
<th>gi accession number</th>
<th>MW (kDa)/pl</th>
<th>Number of peptides matched</th>
<th>% Coverage of matched peptides</th>
<th>Z-score&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prdx 6</td>
<td>16758348</td>
<td>25/5.6</td>
<td>12</td>
<td>63</td>
<td>2.38</td>
</tr>
<tr>
<td>MAWDBP (spot 4112)</td>
<td>19924063</td>
<td>32/6.5</td>
<td>12</td>
<td>44</td>
<td>2.34</td>
</tr>
<tr>
<td>GPx 1</td>
<td>121668</td>
<td>22/7.7</td>
<td>8</td>
<td>61</td>
<td>2.25</td>
</tr>
<tr>
<td>3-α-HSD</td>
<td>19924087</td>
<td>37/6.7</td>
<td>17</td>
<td>60</td>
<td>2.37</td>
</tr>
<tr>
<td>3(2),5-bisphosphate nucleotidase 1</td>
<td>25282455</td>
<td>33/5.6</td>
<td>7</td>
<td>30</td>
<td>2.16</td>
</tr>
<tr>
<td>DNAk-type molecular chaperone</td>
<td>grp 75 precursor</td>
<td>2119726</td>
<td>74/5.9</td>
<td>14</td>
<td>25</td>
</tr>
<tr>
<td>Pre-proalbumin, 608 aa</td>
<td>19705431</td>
<td>69/6.1</td>
<td>33</td>
<td>50</td>
<td>2.40</td>
</tr>
<tr>
<td>MAWDBP (spot 4117)</td>
<td>19924063</td>
<td>32/6.5</td>
<td>9</td>
<td>34</td>
<td>2.24</td>
</tr>
<tr>
<td>Cofilin 1</td>
<td>8393101</td>
<td>19/8.5</td>
<td>6</td>
<td>33</td>
<td>2.09</td>
</tr>
</tbody>
</table>

<sup>1</sup> The proteins are designated with their gi accession number of the NCBI database.

<sup>2</sup> Theoretical MW and pl obtained from the database entry without any processing.

<sup>3</sup> In general, a Z-score $\geq 1.65$ indicates a statistically correct match.

oxidant folate molecules (35,44) per se, to the oxidative stress induced by folate depletion is presently unknown.

Folate deficiency has been associated with increased risk of cancers (8,16). Decreased expression of adhesion molecules and increased expression of urokinase occur in the colon mucosa of folate-deficient rats, suggesting that cell detachment and migration, 2 cancer-related processes, may be modulated by folate status (45). We report here that the liver abundance of cofilin 1, a protein downregulated in highly metastatic hepatocellular carcinoma cells (46), was decreased in FD rats. Cofilin 1 acts as an actin-depolymerizing factor that can control actin-based motility by reversible phosphorylation, generate cell surface protrusions, and set the direction of cell migration (47). Another protein possibly linked to cancer, namely, MAWDBP, was identified in the present study as 2 separate spots with the same molecular weight but different isoelectric point (pl) values, and regulated by folate status. The volume of the protein spot with the highest pl was increased, whereas that of the other spot was almost nil in “FD gels,” compared with “C gels.” This result obtained on silver-stained 2-DE gels was confirmed by Western blotting of similar 2-DE-separated liver protein extracts. The 2-spots/1-spot pattern may be indicative of folate-dependent, post-translational modification (e.g., phosphorylation) of this protein. However, current information on the regulation of MAWDBP expression is scarce. Interestingly, it could interact with MAWD, a protein containing WD-40 repeats that contribute to protein/protein interactions in various cellular processes (48). Overexpression of the MAWD gene in cultured cells causes activation of MAPK, disruption of contact inhibition and anchorage-independent growth (48). The possible existence of 2 phosphorylation states for MAWDBP would be coherent with such a relation between MAWD and MAPK. Folate depletion also gave rise to a 1.5-fold augmentation in the volume of another protein spot that was identified as 3α-HSD, also known as aldo-keto-reductase IC9 (AKR1C9). This change might be related to possible transcriptional activation of the AKR1C gene by reactive oxygen species via an antioxidant responsive element, as observed previously in HepG2 hepatoma cells (49). Such an induction was suggested to exacerbate cellular damage mediated by various xenobiotics and to play a significant role in carcinogenesis.

A reduced abundance of hepatic preproalbumin was also observed in FD rats, suggesting diminished tissue biosynthesis of this protein and subsequent hypoalbuminemia. Hypoalbuminemia is a frequent feature of cachectic patients afflicted with chronic diseases, including cancer and inflammatory disorders (50). Finally, a decreased abundance of 3(2),5-bisphosphate nucleotidase 1 was also determined in the liver of FD rats. This enzyme is important in RNA processing, sulfation-dependent hepatic detoxification, and phosphoinositide-signaling pathway (51).

In conclusion, the present proteomic analysis shows that in adult rats, dietary folate depletion leads to significant changes in the abundance of several liver proteins concurrently with altered folate status and increased homocysteinemia. The results were validated by activity assay and/or Western blot analysis of both GPx 1 and MAWDBP. Most of the proteins identified are related to the control of oxidative stress, inflammatory response, or cancer-related processes, and the changes in their abundance are consistent with the literature. Future studies will aim at determining the regulatory mechanisms, particularly at the level of protein synthesis and degradation, that link the identified proteins to the redox state and the folate status of individuals.

ACKNOWLEDGMENTS

The authors thank I. Pouget and M. Morzel (Station de Recherche sur la Viande, INRA-Theix) for their assistance with the 2-DE gels, J. C. Guillard (General Hospital of Dijon, France) for his help.
with the homocysteine assay, and C. Besson and J. Sauvant (Unité Maladies Métaboliques et Micronutriments, INRA-Theix) for their technical contribution.

LITERATURE CITED