Fructose-3-kinase (FN3K) is an enzyme that appears to be responsible for the removal of fructosamines from proteins. In this study, we report the sequence of human and mouse cDNAs encoding proteins sharing 65% sequence identity with FN3K. The genes encoding FN3K and FN3K-related protein (FN3K-RP) are present next to each other on human chromosome 17q25, and they both have a similar 6-exon structure. Northern blots of mouse tissues RNAs indicate a high level of expression of both genes in bone marrow, brain, kidneys, and spleen. Human FN3K-RP was transfected in human embryonic kidney (HEK) cells, and the expressed protein was partially purified by chromatography on Blue Sepharose. Unlike FN3K, FN3K-RP did not phosphorylate fructoselysine, 1-deoxy-1-morpholino-fructose, or lysozyme glycated with glucose. In a more systematic screening for potential substrates for FN3K-RP, we found, however, that both enzymes phosphorylated ketosamines with a D-configuration in C3 (psicoselysine, 1-deoxy-1-morpholino-psicose, 1-deoxy-1-morpholino-ribulose, lysozyme glycated with allose—the C3 epimer of glucose, or with ribose). Tandem mass spectrometry and nuclear magnetic resonance analysis of the product of phosphorylation of 1-deoxy-1-morpholino-psicose by FN3K-RP indicated that this enzyme phosphorylates the third carbon of the sugar moiety. These results indicate that FN3K-RP is a ketosamine-3-kinase (ketosamine-3-kinase 2). This enzyme presumably plays a role in freeing proteins from ribulosamines or psicosamines, which might arise in a several step process, from the reaction of amines with glucose and/or glycolytic intermediates. This role is shared by fructose-3-kinase (ketosamine-3-kinase 1), which has, in addition, the unique capacity to phosphorylate fructosamines. *Diabetes* 52:2888–2895, 2003

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Primary amines of proteins and other compounds spontaneously react with carbohydrates that possess a free carbonyl group to produce Schiff bases. These slowly rearrange to form Amadori or Heyns products, depending on whether the reacting sugar was an aldo or a ketose (1–4). This process, known as nonenzymatic glycation, is best known for glucose, leading then to the production of fructosamines. Similar reactions occur with other sugars (mannose, galactose, fructose, and pentoses) (5) and phosphorylated sugar derivatives (6–8). In fact, glucose is intrinsically one of the least reactive sugars in this respect because it is well stabilized in its hemiacetal form (5).

Formation of fructosamines has attracted much attention in the context of diabetes. This is because the glycation rate is first order with respect to glucose concentration. Serum fructosamines and glycated hemoglobin are therefore assayed to assess the blood glucose concentration in the preceding weeks or months (9–11). Furthermore, fructosamines are thought to participate in the pathogenesis of long-term diabetes complications by acting either as such (12) or after conversion to advanced glycation end products (13–15).

The sequence of a mammalian protein that catalyzes the phosphorylation of low–molecular weight and protein-bound intracellular fructosamines on the third carbon of their deoxyfructose moiety has recently been reported (16,17). Because fructosamine 3-phosphate residues are unstable (17), fructosamine-3-kinase (FN3K) appears to cause the removal of fructosamine residues from proteins. Accordingly, 1-deoxy-1-morpholino-fructose, a (substrate and) competitive inhibitor of FN3K, caused a twofold increase in the accumulation of glycated hemoglobin in erythrocytes incubated with 200 nmol/l glucose (18).

While cloning the cDNA encoding FN3K, we noted the existence of human and mouse cDNAs encoding a related protein. In this study, we report the sequence of this FN3K-related protein (FN3K-RP) and the identification of its biochemical function.

RESEARCH DESIGN AND METHODS

Preparation of ketosamines. Psicoselysine was synthesized from allose and t-BOC-lysine and purified as previously described for fructoselysine (18,19).

1-Deoxy-1-morpholino-psicose, 1-deoxy-1-morpholino-ribulose, and 1-deoxy-
1-morpholino-xylulose were synthesized from morpholine and allulose, ribose, or xylose, respectively (20). The incubation time after the addition of ethanol was reduced to 15 min in the last two cases. The products were purified on AG50W-X8 (H^+ form) columns from which FN3K-RP and FN3K were eluted as a single major peak. They were desalted by gel filtration on Biogel P2 and quantified by measuring the reducing power (21), using 1-deoxy-1-morpholino-fructose as a standard. Mass spectrometry analysis indicated that the products had the expected m/z ratio. Their purity was checked by paper chromatography on 3 MM paper in two different solvents (ethyacetate/pyridine/water: 12/5/4 and ethylacetate/water/acetic acid/formic acid: 18/4/5/1) and estimated to be >98% for pure 1-deoxy-1-morpholino-psicose, and 1-deoxy-1-morpholino-xylulose, and to ~70% for 1-deoxy-1-morpholinosibulose. Using this method, we could ascertain that psicoselysine and 1-deoxy-1-morpholinosicose were not contaminated with their C3 epimers, which had distinct Rfs. 1-Deoxy-1-morpholinosibulose appeared to be contaminated with ~20% 1-deoxy-1-morpholinoxylosulose.

\[^{14}C\]1-deoxy-1-morpholino-sibulose was synthesized by incubating 12.5 \( \mu \)Ci [1-\(^{14}\)C]ribose (American Radiochemical) with 30 \( \mu \)l morpholine at 70°C, 8 \( \mu \)l acetic acid and 40 \( \mu \)l ethanol were added after 30 and 40 min, respectively, and the incubation pursued for 15 min. Further purification on the cation exchanger (see above) resulted in a product that could be phosphorylated by FN3K or FN3K-RP to an extent of ~50%. A more pure radiolabeled product was obtained by purifying this product of phosphorylation with FN3K (obtained after a 40-min incubation at 30°C in the presence of 1.2 nM FN3K, 25 mmol/l Tris, pH 7.8, 1 mmol/l MgCl\(_2\), 1 mmol/l dithiothreitol, 50 mmol/l ATP-Mg, and 500,000 cpm [\(^{32}\)P]ATP in a final volume of 60 \( \mu \)l. The reaction was stopped by adding 90 \( \mu \)l ice-cold 10% (w/v) perchloric acid. After neutralization with K\(_2\)CO\(_3\), the supernatants were diluted to 1 ml with 20 mmol/l MES, pH 6, and loaded onto anion-exchange columns (AG1X8, Cl\(^{-}\) form, 1 ml). These were then washed with five volumes of 20 mmol/l MES, pH 6, to elute the phosphorylated Amadori compound, the unreacted [\(^{32}\)P]ATP remaining bound to the column. The eluate was mixed with 15 ml OptimaGold (Packard) scintillation fluid and counted for radioactivity. Phosphorylation of glycated lysyosine was assayed by incorporation of [\(^{32}\)P]ATP (16) in a mixture containing 25 mmol/l Tris, pH 7.8, 1 mmol/l EGTA, 1 mmol/l MgCl\(_2\), 1 mmol/l dithiothreitol, 2.1 mg/ml glycated human egg lysozyme, and 100 mmol/l ATP-Mg. Phosphorylation of 1-deoxy-1-morpholinosibulose was measured using [\(^{32}\)P]1-deoxy-1-morpholinosibulose, as previously described for [\(^{34}\)C]1-deoxy-1-morpholinosibulose (16).

Preparation of phosphorylated 1-deoxy-1-morpholinosibulose for nuclear magnetic resonance and mass spectrometry analysis. 1-Deoxy-1-morpholinosibulose (25 mmol/l) was incubated for 18 h at 30°C in a final volume of 2 ml in the presence of 25 nM FN3K, 7.5 mmol/l ATP-Mg, and 500,000 cpm [\(^{32}\)P]dCTP by random priming. Measurement of enzymatic activity. Phosphorylation of 1-deoxy-1-morpholinosibulose, 1-deoxy-1-morpholinosicose, and psicoselysine was assayed at 30°C in a mixture containing 25 mmol/l Tris, pH 7.8, 1 mmol/l EGTA, 1 mmol/l MgCl\(_2\), 1 mmol/l dithiothreitol, 50 mmol/l ATP-Mg, and 500,000 cpm [\(^{32}\)P]ATP in a final volume of 60 \( \mu \)l. The reaction was stopped by adding 90 \( \mu \)l ice-cold 10% (w/v) perchorlic acid. After neutralization with K\(_2\)CO\(_3\), the supernatants were diluted to 1 ml with 20 mmol/l MES, pH 6, and loaded onto anion-exchange columns (AG1X8, Cl\(^{-}\) form, 1 ml). These were then washed with five volumes of 20 mmol/l MES, pH 6, to elute the phosphorylated Amadori compound, the unreacted [\(^{32}\)P]ATP remaining bound to the column. The eluate was mixed with 15 ml OptimaGold (Packard) scintillation fluid and counted for radioactivity. Phosphorylation of glycated lysyosine was assayed by incorporation of [\(^{32}\)P]ATP (16) in a mixture containing 25 mmol/l Tris, pH 7.8, 1 mmol/l EGTA, 1 mmol/l MgCl\(_2\), 1 mmol/l dithiothreitol, 2.1 mg/ml glycated human egg lysozyme, and 100 mmol/l ATP-Mg.

For the synthesis of glycated lysyosine, a solution containing 60 mg/ml hen egg lysozyme, 25 mmol/l HEPES, pH 7.1, and 1 mol/l of the indicated aldoses was filtered on a 0.22-\( \mu \)m membrane and incubated at 37°C for 20 days (alone and glucose) or 3 days (ribose). Glycated lysyosine was purified by gel filtration on a Biogel P2 column equilibrated with water. The degree of glycation, estimated by nanoelectrospray mass spectrometry, was 1.35 mol/mol. Measurement of enzymatic activity. Phosphorylation of 1-deoxy-1-morpholinosibulose, 1-deoxy-1-morpholinosicose, and psicoselysine was assayed at 30°C in a mixture containing 25 mmol/l Tris, pH 7.8, 1 mmol/l EGTA, 1 mmol/l MgCl\(_2\), 1 mmol/l dithiothreitol, 2.1 mg/ml glycated hen egg lysozyme, and 100 mmol/l ATP-Mg. Phosphorylation of 1-deoxy-1-morpholinosibulose was measured using [\(^{32}\)P]1-deoxy-1-morpholinosibulose, as previously described for [\(^{34}\)C]1-deoxy-1-morpholinosibulose (16).

Preparation of phosphorylated 1-deoxy-1-morpholinosibulose for nuclear magnetic resonance and mass spectrometry analysis. 1-Deoxy-1-morpholinosibulose (25 mmol/l) was incubated for 18 h at 30°C in a final volume of 2 ml in the presence of 25 nM FN3K, 7.5 mmol/l ATP-Mg, and 500,000 cpm [\(^{32}\)P]dCTP by random priming. Standard Bruker pulse programs were used to obtain one-dimensional phosphorus spectra (with and without proton decoupling), proton spectra (with and without phosphorus decoupling), \(^{1}H\)-\(^{13}C\) correlation spectra (COSY), and \(^{1}H\)-\(^{31}P\) spectra (heteronuclear multiple bond connectivity (HMBCC)). A delay of 50 ms was used for evolution of \(^{3}J_{\text{HP}}\) constants. \(^{31}P\) chemical shifts were referenced to external 85% \( \text{H}_2\text{PO}_4\) and proton chemical shifts and to 3-(trimethylsilyl)propanesulfonic acid (sodium salt).
differed from each other through their 3' untranslated region, of 812 and 735 bp, respectively, and by two single nucleotide changes at positions 1433 and 1555.

The mouse cDNA sequence encoding FN3K-RP was established by 5' and 3' RACE experiments starting from EST sequence AI647859, which contains nucleotides 445–646 of the final cDNA sequence. The sequence that we obtained contained 48 bp of the 5' untranslated region and 78 bp of the 3' untranslated region (whose length is 2 kb, see below). It encoded a protein identical to hypothetical protein XP-137924, which in the meantime had appeared in databanks.

The predicted human and mouse FN3K-RPs have the same length (309 amino acids) as human and mouse FN3Ks (Fig. 1). They share 88% sequence identity with each other and 64% sequence identity with human and mouse FN3Ks. Like FN3Ks, their second amino acid is a glutamate, which suggests that their NH2-terminal residue is an acetylated methionine (27), as is the case for FN3K (16). They are homologous to bacterial proteins of unknown function, with which they share 25–35% sequence identity (not shown). Like FN3K (16), FN3K-RP comprises an HGDxxxxN motif also found in aminoglycoside kinases.

Gene structure and chromosomal localization of FN3K and FN3K-RP. Basic local alignment search tool searches with the human genome sequences indicate that FN3K-RP is located in the telomeric region of the long arm of chromosome 17, next to the gene encoding FN3K (not shown). Both genes are separated by ~2 kb, with which they share 55–35% sequence identity (not shown). Like FN3K (16), FN3K-RP comprises an HGDxxxxN motif also found in aminoglycoside kinases.

Two FN3K pseudogene fragments were identified on chromosome 22. One corresponds to exon 2 flanked by parts of introns 1 and 2 and the other to exon 3, intron 3, and exon 4, with adjacent parts of introns 2 and 4. They both share >95% identity with the human FN3K gene, indicating recent duplication events of portions of the FN3K gene.

As Szwergold, Howell, and Beisswenger (17) reported the localization of the FN3K gene on chromosome 1, we checked the chromosomal localization by fluorescence in-situ hybridization (FISH). For this, a PAC clone containing the FN3K gene (no. 127J18) was identified on human RPCI-1 PAC filters (28) by hybridization of a human FN3K probe. PCR amplification of introns using exonic primers showed that this clone contained both FN3K and FN3K-RP genes (not shown). FISH analysis indicated that it hybridized with the telomeric region of chromosome 17 (Fig. 2). No signal was detected on chromosome 1.

Tissular distribution of FN3K and FN3K-RP. Northern blots (Fig. 3) showed that the mouse FN3K mRNA is about 1.1 kb in length, which is in agreement with the published sequence (16), whereas the FN3K-RP mRNA is much longer, indicating the presence of an ~2 kb 3' untranslated region. Both mRNAs show the highest levels of expression in bone marrow, brain, spleen, kidney, and lens (allowing for the lower amount of RNA loaded onto the gels in the latter case). Specific expression of FN3K-RP was also observed in testis, thymus, and lungs. Both FN3K and FN3K-RP are also expressed at lower levels in heart, liver, and skeletal muscle. No expression was observed in the intestinal mucosa.

Overexpression and partial purification of FN3K-RP. Attempts were made to express both human and mouse FN3K-RPs in E. coli using pET3a as a vector and E. coli BL21pLysS as host cells. In the case of mouse FN3K-RP, SDS-PAGE analysis indicated that an insoluble protein...
with the expected mass (~34 kDa) was obtained. This protein cross-reacted with rabbit polyclonal antibodies raised against human FN3K, with an approximately threefold lower reactivity than human FN3K (not shown). No expression of human FN3K-RP could be detected.

We therefore resorted to expression in eukaryotic cells. Extracts of HEK cells that had been transfected with plasmids driving the expression of human FN3K or human FN3K-RP were chromatographed on Blue Sepharose, which is known to strongly retain FN3K (16). Proteins were eluted from the column with a salt gradient. Analysis of the fractions by Western blotting with anti-FN3K antibodies indicated the presence of FN3K in the fractions eluted with a high salt concentration from the column loaded with the extracts of cells expressing human FN3K (Fig. 4A). A protein eluting with lower salt concentrations was detected with the same antibodies in the fractions of the column loaded with FN3K-RP (Fig. 4B). None of these proteins could be detected in fractions of a column on which an extract of cells transfected with a control plasmid had been loaded (not shown). These results indicated that soluble FN3K-RP was expressed in HEK cells and that this protein had a distinct chromatographic behavior as compared with FN3K. Coomassie Blue staining of SDS-PAGE gels indicated that this protein accounted for ~10% of total protein in fractions 4 to 6 of the column shown in Fig. 4A.

**Enzymatic activity of FN3K-RP.** As shown in Fig. 4A, overexpressed FN3K catalyzed the phosphorylation of protein-bound fructosamines, whereas no such activity was observed with FN3K-RP. We therefore undertook a more systematic screening of ketosamines as potential substrates of FN3K-RP (and of FN3K) and found that both enzymes catalyzed the phosphorylation of proteins that had been glycated with allose and which therefore contained “psicosamines.” In the case of FN3K, the kinase activity observed was about 1.6-fold higher with D-alllose-glycated lysozyme than with glucose-glycated lysozyme, but this is presumably due to the higher glycation extent observed in the former than in the latter case (3.3 vs. 1.3 mol/mol). Both proteins were also found to phosphorylate lysozyme that had been glycated with D-ribose (results not shown).

These findings prompted us to study the phosphorylation of low–molecular weight psicosamines and ribulosamines. Both FN3K and FN3K-RP were found to phosphorylate 1-deoxy-1-morpholino-psicose, psicoselysine, and 1-deoxy-1-morpholino-ribulose, but only FN3K phosphorylated 1-deoxy-1-morpholino-fructose (Table 1) and fructoselysine (not shown). With both enzymes, the $K_M$ values for the model ribulosamine were 40- to 80-fold lower than for the low–molecular weight psicosamines, but only about 3- to 4-fold lower than the $K_M$ for 1-deoxy-1-morpholino-fructose in the case of FN3K. Furthermore, the latter enzyme displayed an ~10-fold higher $V_{max}$ with 1-deoxy-1-morpholino-fructose than with 1-deoxy-1-morpholino-ribulose.

We also tested the ability of the low–molecular weight ketosamines to inhibit the phosphorylation of lysozyme glycated with allose or with ribose. When the substrate was lysozyme glycated with allose, FN3K was inhibited...
### TABLE 1

Kinetic properties of FN3K and FN3K-RP

<table>
<thead>
<tr>
<th>Compounds</th>
<th>FN3K (ketosamine 3-kinase 1)</th>
<th>FN3K-RP (ketosamine 3-kinase 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_M$ (µmol/l)</td>
<td>$V_{max}$ (mU/mg)</td>
</tr>
<tr>
<td>Deoxymorpholino-fructose</td>
<td>10</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n = 3)</td>
</tr>
<tr>
<td>Deoxymorpholino-psicose</td>
<td>160</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n = 4)</td>
</tr>
<tr>
<td>Psicoselysine</td>
<td>140</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n = 4)</td>
</tr>
<tr>
<td>Deoxymorpholino-ribulose</td>
<td>$2.6 \pm 0.1$</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>(n = 4)</td>
<td></td>
</tr>
</tbody>
</table>

Data are the means of two values or means ± SE. For both enzymes, the first two columns indicate the $K_M$ and $V_{max}$ values for the indicated compounds. The third and fourth columns show the concentrations of compounds needed to cause 50% inhibition of the enzymatic activity ($I_{50}$) determined on lysozyme glycated with allose or with ribose or the inhibition reached at the indicated concentration. The preparation of enzymes used were purified recombinant human FN3K expressed in *E. coli* (16) and FN3K-RP purified from HEK-293 cells. For the calculation of the $V_{max}$, the concentration of FN3K or FN3K-RP was estimated from SDS-PAGE gels that had been stained with Coomassie Blue by comparison with standards.

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**FIG. 5.** Tandem mass spectrometry analysis of phosphorylated 1-deoxy-1-morpholino-fructose. The tandem mass spectrometry analysis of the phosphorylated 1-deoxy-1-morpholino-fructose, the major anion of m/z 328.1, which corresponds to the mass fragmentation of 1-deoxy-1-morpholino-fructose-phosphate. The ion at m/z 310 is most likely a dehydration product. The peak at m/z 282 is shown. The ion at m/z 310 and 328 are the two major anions.
as well as 1H-31P-HMBC spectra (Fig. 6), were acquired to phosphorus broadband decoupling, resonance in COSY. This conclusion was corroborated by position 3 followed from the observation that the psicose moiety was coupled to phosphorus, proton-proton shifts at pH 6.0 were 5.56, 3.97, 3.92, and 3.64 ppm, the chemical structures with (not shown) and without phosphorus, proton-proton COSY were acquired. The assignment of protons bound to position 3 followed from the observation that the corresponding resonances correlated only with one other resonance in COSY. This conclusion was corroborated by the observation that the proton multiplets in the 1H-spectrum collapsed to doublet resonances when phosphorus decoupling was applied (not shown).

**DISCUSSION**

**Enzymatic activity of FN3K-RP and FN3K.** The new enzyme that we describe in the present study phosphorylates psicosamines and ribulosamines, though not fructosamines. The phosphorylated carbon could be identified as C3 by analysis of the phosphorylation product of 1-deoxy-1-morpholino-psicose-phosphate. It is most likely that the same carbon is phosphorylated in other substrates, particularly because the homologous enzyme FN3K also phosphorylates the third carbon of the sugar portion of its substrates. FN3K-RP therefore appears to be a ketosamine-3-kinase with a distinct substrate specificity as compared with FN3K.

Another new finding reported in the present article is that FN3K has a much broader substrate specificity than previously thought because it acts not only on fructosamines but also on other ketosamines, with comparable (ribulosamines) or significantly lower affinities (psicosamines and maybe also tagatosamines, which were found to act as inhibitors) than it displays for fructosamines. This enzyme is therefore also a ketosamine-3-kinase. It is, however, the only enzyme that acts on fructosamines, which are probably quantitatively the most important ketosamines that are formed under physiological conditions. Furthermore, it displays a higher catalytic efficiency ($V_{\max}/K_M$ ratio) on a model fructosamine (1-deoxy-1-morpholino-fructose) than on the equivalent ribulosamine (1-deoxy-1-morpholino-ribulose). Therefore, its name “fructosamine-3-kinase” appears justified, although its designation as “ketosamine-3-kinase 1” and that of FN3K-RP as “ketosamine-3-kinase 2” would be more rigorous. For the sake of clarity, the names FN3K and FN3K-RP will be used in the rest of the DISCUSSION.

**Chromosomal localization and tissular distribution.** The gene encoding FN3K-RP is located next to the one encoding FN3K in both the human and mouse genomes, indicating an ancestral duplication event. The localization on human chromosome 17, as now reported in databases, was confirmed by in-situ hybridization with a PAC clone containing both genes. The previous localization of the FN3K gene on chromosome 1 (17) is most likely due to uncertain assignment of some PAC clones in an earlier phase of the human genome sequencing project.

Previous RT-PCR experiments performed on human RNAs indicated a widespread tissular distribution of FN3K, with particularly high levels in kidney (17). The Northern blots performed on mouse tissues in the present study indicate a wide variability of expression level from tissue to tissue. The most intense signals were observed with RNA from bone marrow, brain, and kidney, whereas other organs such as liver, intestinal mucosa, testis, thymus, and lung have no or barely detectable mRNA. It is not known at present if this difference in tissular distribution is due to a species difference or to a difference in the technique that was used.

**Physiological function.** Recent work indicates that the function of FN3K is most likely to remove fructosamine residues from proteins (17,18). Fructosamine 3-phosphates are indeed unstable and decompose slowly to 3-deoxyglucosone, inorganic phosphate, and an amine (18). FN3K therefore appears to be a protein-repair enzyme. Such enzymes are expected to play an important role in tissues that contain proteins with long (half-)lives. This is certainly the case of erythrocytes and lenses (29), but it is also true for brain, where myelin proteins have a half-life much longer than 10 days (30). Interestingly, the knockout of another protein-repair enzyme, isoaspartyl-methyltransferase (31), resulted in intractable seizures, leading to death of the mice after a few weeks. This result underlines the importance of protein-repair mechanisms in the brain and is consistent with the finding of elevated mRNA levels for both FN3K and FN3K-RP in this tissue.

As all ketosamine 3-phosphates other than fructosamine 3-phosphates are most likely also unstable, FN3K-RP could also potentially play the role of a deglycating enzyme. The most puzzling question is that of the identity of the substrates upon which this enzyme is acting physio-

![FIG. 6. 1H-31P correlation spectrum through multiple-bond coupling (HMBC) of synthesized 1-deoxy-1-morpholino-psicose-phosphate. Spectra were run on a Bruker DRX500 at a probe head temperature of 30°C. The pH value of the solution was 6.0. The proton resonances assigned to H_y in the four forms of the metabolite are labeled in the proton spectrum. The one-dimensional, proton-coupled 31P spectrum is also represented.](image-url)
logically. Its best in vitro substrates are two compounds with a hydroxyl group in the D configuration on C3 (D-ribulosamines and D-psicoamines), whereas it displays little if any affinity for ketosamines with an L configuration in C3. Fructosamines are indeed neither substrates nor inhibitors of FN3K-RP, and a tagatosamine and a xylosamine were found to be poor inhibitors of this enzyme.

The question then is to know if and how these substrates could arise in vivo. As allose is not a physiological sugar, it cannot be a source of psicosamines. These could, however, arise through epimerization of fructosamines or of an intermediate in the formation of Amadori products from glucose (Fig. 7). Preliminary data indicate that when proteins are incubated with elevated concentrations of glucose in the presence of 200 mmol/l inorganic phosphate, which facilitates the formation of fructosamines (32), a substrate of FN3K-RP forms (F.C., E.V.S., unpublished data), suggesting that epimerization around the third carbon of fructosamines takes place under these conditions.

Ribulosamines could hypothetically form either directly through reaction of amines with D-ribose or indirectly through reaction with ribose 5-phosphate (Fig. 7) or ADP-ribose, followed by (enzymatic?) removal of phosphate or ADP. Little is known about the concentration of free ribose, ribose 5-phosphate, and ADP-ribose in most cell types. It is, however, worth mentioning that the latter two are extremely powerful glycating agents (7,8) and that it would therefore make sense to have an enzymatic system able to remove the corresponding Amadori products from proteins. As hyperglycemia stimulates the activity of the pentose phosphate pathway (33), poor glycemic control could also favor glycation by ribose 5-phosphate, further linking the new enzyme with diabetes.

Further evidence for a role of the new enzyme in combating glucose-induced glycation is to be found in the observation that the incubation of erythrocytes with an elevated glucose concentration under the conditions previously described (18) leads to the formation of substrates not only for FN3K but also FN3K-RP (G.D., F.C., J. Fortpied, and E.V.S., unpublished data). The identity of the FN3K-RP substrates formed under these conditions still remains to be determined; psicosamines and ribulosamines are both likely candidates.

Compared with FN3K-RP, FN3K has the unique capacity to phosphorylate fructosamines, suggesting that one of its important functions is to repair damages provoked by glucose. As both ketosamine-3-kinases phosphorylate psicosamines and ribulosamines with similar affinities, one may wonder if FN3K-RP has any specific role to play. One possibility is that this enzyme is able to phosphorylate as yet unknown substrates that are not utilizable by FN3K. In addition, it should be stressed that FN3K-RP appears to be expressed in tissues (testis and lung) where the FN3K mRNA is undetectable. In conclusion, the data reported in the present article indicate the existence of an enzyme that may complement the action of FN3K in a putative deglycation process.

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REFERENCES