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## PROTEIN REPAIR AND INBORN ERRORS OF METABOLISM

*Our laboratory has a longstanding interest in the metabolism of carbohydrates and related compounds. The study of the mechanism of formation of an intriguing phosphate ester, fructose 3-phosphate, led us to identify fructosamine 3-kinase, an enzyme serving to remove sugar adducts from proteins. Other enzymes are potentially implicated in protein deglycation and we try to understand their role. Our group aims also at identifying enzymes that are potentially implicated in inborn errors of metabolism.*

### PROTEIN DEGLYCATION

*J. Fortpied, M. Veiga-da-Cunha, Y. Achouri, E. Van Schaftingen*

Chronic elevation of the blood glucose concentration in diabetes appears to be responsible for the long-term complications of this disease. The link between the elevated concentration of glucose and the development of these complications is not clear. One of the theories on this link emphasizes the role of fructosamines. These are formed through a spontaneous reaction (known as 'glycation') of glucose with primary amines, followed by

an Amadori rearrangement. Fructosamine 3-kinase (FN3K) is a recently identified enzyme that phosphorylates both low-molecular-weight and protein-bound fructosamines (3). Fructosamine 3-phosphates are unstable, breaking down spontaneously to 3-deoxyglucosone, inorganic phosphate and the amino compound that originally reacted with glucose (Fig. 1).

That FN3K indeed acts as a 'deglycating' enzyme was first indicated by experiments in which erythrocytes were incubated *ex vivo* with an elevated concentration of glucose, with or without a competitive inhibitor of FN3K. These studies showed also that only part of

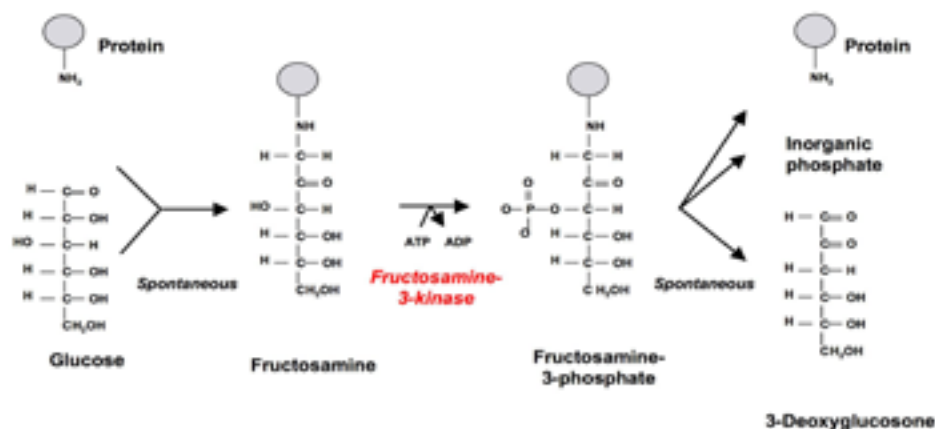


Figure 1. Formation and breakdown of fructosamines

the fructosamines—those that are accessible to FN3K—are cleared in this way. The role of FN3K in deglycation was confirmed and extended by analysis of a mouse FN3K knockout model that we created (8). Protein-bound fructosamines are increased by a factor of about 2 in FN3K-deficient mice. This applies only to intracellular proteins, consistent with the fact that FN3K is a cytosolic enzyme.

We have also identified several other enzymes that are potentially involved in protein deglycation. A first one is fructosamine-3-kinase related protein (FN3K-RP; ref 2). This enzyme shares about 65 % sequence identity with FN3K and is encoded by a gene that is present next to the FN3K gene on human chromosome 17q25. A similar gene arrangement is found in other mammals and in chicken, although not in fishes, indicating that a gene duplication event occurred during or after the fish radiation. FN3K-RP is also a ketoamine 3-kinase, acting best on ribulosamines and erythrusamines, but not at all on fructosamines. An enzyme with a similar substrate specificity is found in many fishes, in plants and in a significant proportion ( $\approx 25\%$ ) of bacteria. All ketoamine 3-phosphates are unstable and their spontaneous decomposition regenerates the free amino group, indicating that FN3K-RP is also a protein repair enzyme.

It is unlikely that the physiological substrates of FN3K-RP are formed through a reaction of amines with free ribose or erythrose, because these sugars are present at very low concentrations ( $< 10\ \mu\text{M}$ ) in tissues. They are most likely formed through a reaction of proteins with ribose 5-phosphate or erythrose 4-phosphate, two extremely potent glycation agents that react  $\approx 80$  and 500-fold more rapidly than glucose. The ribulosamine 5-phosphates (Fig. 2) and erythrusamine 4-phosphates that are formed from phosphorylated intermediates need to be dephosphorylated before being phosphorylated on their third carbon by FN3K-RP, and thereby destabilized and removed from proteins. The phosphatase catalyzing this reaction has recently been identified as LMW-PTP (low-molecular-weight protein-tyrosine-phosphatase). One of our goals is to understand the physiological significance of FN3K-RP-mediated deglycation.

## NEUROMETABOLIC DISORDERS

*Y Achouri, G. Noël, M. Veiga da Cunha, E. Wiame, E. Van Schaftingen*

D- and L-2-hydroxyglutaric acidurias are

distinct neurometabolic diseases characterized by the accumulation of abnormal amounts of either D- or L-2-hydroxyglutarate in cerebrospinal fluid, blood and urine. Work in our lab has led to the elucidation of the metabolism of these compounds (Fig. 2). Both of them are converted to alpha-ketoglutarate by distinct FAD-linked dehydrogenases. The dehydrogenase acting on L-2-hydroxyglutarate is bound to mitochondrial membranes and mutations in its gene are found in virtually all cases of L-2-hydroxyglutaric aciduria (7). The dehydrogenase acting on D-2-hydroxyglutarate is in the mitochondrial matrix and most likely transfers its electrons to the respiratory chain via electron-transfer-flavoprotein (1). It is mutated in about 40 % of the patients with D-2-hydroxyglutaric aciduria.

tand the physiopathological mechanisms of this disease.

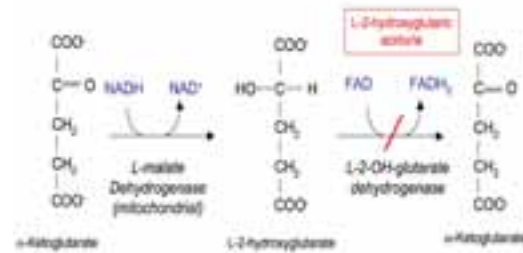


Figure 3. Formation and breakdown of L-2-hydroxyglutarate

## FRUCTOSAMINES, MANNOSE BINDING LECTIN AND DIABETES COMPLICATIONS

*J. Fortpied, E. Van Schaftingen, in collaboration with Didier Vertommen, Horm Unit*

Complement activation via the mannose-binding lectin (MBL) pathway has been proposed to play a role in the pathogenesis of diabetic vascular complications. Since protein glycation is increased in diabetes, we tested the possibility that the glycation product fructoselysine is a ligand for MBL and that its interaction with this protein may initiate complement activation.

We investigated the binding of MBL to fructoselysine by chromatography of human serum on fructoselysine-Sepharose, followed by Western blot and mass spectrometry analysis. We also performed ELISA assays using purified MBL and fructoselysine-derivatized (binding assay) or mannan-coated plates (inhibition assay). Complement activation was determined by the fixation of C3d following incubation of fructoselysine-derivatized plates with serum from subjects producing different levels of MBL.

MBL and its associated proteases were selectively purified from serum by chromatogra-

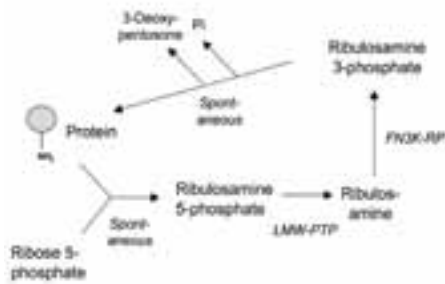


Figure 2. Formation and breakdown of ribulosamines

The formation of L-2-hydroxyglutarate is catalyzed by mitochondrial L-malate dehydrogenase (Fig. 3). This enzyme is not completely specific for oxaloacetate : it also reduces, at a very low rate, alpha-ketoglutarate to L-2-hydroxyglutarate. This activity is sufficient to account for the daily formation of L-2-hydroxyglutarate. Since L-2-hydroxyglutarate does not appear to have any role, but to have only toxic effects, L-2-hydroxyglutarate dehydrogenase is a 'repair enzyme' and L-2-hydroxyglutaric aciduria is a disorder of metabolite repair. One of our aims is to produce a mouse model of L-2-hydroxyglutaric aciduria in order to confirm the origin of L-2-hydroxyglutarate and unders-

phy on fructoselysine-Sepharose. Competition experiments indicated that MBL had a similar affinity for mannose, fructose and fructoselysine. MBL bound, in a highly cooperative manner, to fructoselysine-derivatized plates. This binding was associated with complement activation and was much lower with serum from subjects with low MBL genotypes. From this we could conclude that MBL binding to fructoselysine and the ensuing complement activation may provide a physiopathological link between enhanced glycation and complement activation in diabetes. The cooperative character of this binding may explain the high sensitivity of diabetic complications to hyperglycemia (5).

## IDENTIFICATION OF ENZYMES POTENTIALLY IMPLICATED IN METABOLIC DISEASES

*F. Collard, J. Drozdzak, S. Jaisson, G. Tabay, E. Wiame, G. Connerotte, K. Peel, E. Van Schaftingen, M. Veiga-da-Cunha*

### Synthesis of N-acetyl-aspartate

The brain-specific compound NAA (N-acetylaspartate) is the second most abundant organic molecule in brain. It occurs almost exclusively in neurons, where its concentration reaches  $\approx 20$  mM. Its abundance is determined in patients by MRS (magnetic resonance spectroscopy) to assess neuronal density and health. The molecular identity of the N-acetyltransferase that catalyses NAA synthesis has remained unknown, because the enzyme is membrane-bound and difficult to purify.

Using a database search approach we have identified its gene. Briefly, we have searched the human and mouse genome for putative N-acetyltransferases that would be membrane-bound and exclusively expressed in brain. Two candidates were selected in this way (NAT8L

and NAT14). They were expressed in HEK cells and NAT8L was shown to be the N-acetylaspartate-producing enzyme (10). A patient deficient in N-acetylaspartate was shown to have a homozygous 19 bp deletion in the coding sequence of the NAT8L gene, further proving that NAT8L is responsible for NAA production. In collaboration with P. Courtoy, we also showed that this enzyme is associated with the endoplasmic reticulum, and not with mitochondria, as often stated previously. The molecular identification of this enzyme will lead to new perspectives in the clarification of the function of this most abundant amino acid derivative in neurons and for the diagnosis of hypoacetylaspartia in other patients.

### Formation of mercapturic acids

NAT8 shares about 30 % identity with NAT8L (aspartate N-acetyltransferase). It is expressed in kidney and in liver. We could show that this enzyme corresponds to the acetyltransferase that makes mercapturic acid (N-acetylcysteinyl-S-conjugates), catalysing thereby the last step in one of the major pathways of xenobiotic metabolism (9). In collaboration with Donatienne Tyteca and Pierre Courtoy (Cell Unit), we found that like NAT8L, NAT8 is associated with the endoplasmic reticulum thanks to a non-classical targeting signal that we are now characterizing. NAT8 has recently been shown to be associated with chronic kidney diseases (Chambers et al. Nature Genetics, 2010). Because of the toxicity of non acetylated cysteinyl-S-conjugates, our identification provides a potential explanation for this association. In relation with the metabolism of xenobiotic, we also carried out for the first time the molecular identification of omega amidase, the enzyme that hydrolyzes alpha-ketoglutarate, a product made by transaminases using glutamine as an alpha-amino group donor.

## Carnosine synthase

Carnosine (beta-alanyl-L-histidine) is a most abundant (concentration  $\approx 10$  mM) dipeptide in muscle whereas homocarnosine (gamma-aminobutyryl-L-histidine) is the second most abundant dipeptide in brain of most vertebrates and some invertebrates. Their function is still not well established and the enzyme (carnosine synthase) that synthesizes them both was not well characterized and its molecular identity was unknown. To determine this identity, we have purified carnosine synthase from chicken pectoral muscle (4). We found that this enzyme hydrolyses ATP to ADP and inorganic phosphate and not to AMP and pyrophosphate, as previously assumed. Furthermore, by combining a database mining approach with a mass spectrometry analysis of the purified protein, we could show that carnosine synthase corresponds to a protein of unknown function named ATPGD1 in the databases. This was confirmed by expression and purification of human and mouse ATPGD1, which we found to catalyze the synthesis of both carnosine and homocarnosine (4). The identification of the gene encoding carnosine synthase will help getting a better understanding of the biological functions of carnosine and related dipeptides. Furthermore, it opens the perspective of testing if the low homocarnosine level found in the CSF of some patients is due to primary carnosine/homocarnosine synthase deficiency.

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