

Summary

The spontaneous, non-enzymatically catalysed reaction of glucose with amino-groups of proteins leads to the formation of fructosamines. This process, called *protein glycation*, is slow, nearly irreversible and proportional to glucose concentration, which makes that it is particularly favoured in tissues or cells with a high intracellular glucose concentration and a low or absent protein turnover, like human erythrocytes. Before the beginning of the present work, the only known fate for fructosamines was their spontaneous conversion to a variety of compounds called *advanced glycation end-products*, which are believed to play a role in the development of diabetic complications.

We have identified in human erythrocytes an enzyme that phosphorylates fructosamines on the third carbon of their deoxyfructose moiety. This fructosamine 3-kinase has been purified to near-homogeneity and partially sequenced by tandem mass spectrometry. The sequence information allowed us to clone the cDNA of the human and mouse enzymes, and overexpress these proteins in *Escherichia coli* to study their kinetic properties. These enzymes act not only on low-molecular-weight, but also on protein-bound fructosamines.

The physiological role of fructosamine 3-kinase has been investigated by incubating human erythrocytes in the presence of high concentrations of glucose and of a specific inhibitor of this enzyme. We show that fructosamine 3-kinase converts glycated haemoglobin to a form of haemoglobin with alkali-labile phosphate, presumably corresponding to fructosamine 3-phosphate residues. This phosphorylation step triggers the spontaneous decomposition of fructosamine 3-phosphate residues to free amine, inorganic phosphate and 3-deoxyglucosone, which can be oxidised in the red blood cell to 2-keto-3-deoxygluconate. Fructosamine 3-kinase thus initiates a mechanism of protein *deglycation* in human erythrocytes.