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Transglutaminases

Family of Enzymes with Diverse Functions

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This collection of articles is published as we approach the 50th anniversary of the discovery of the first of a large family of protein-remodelling enzymes by Heinrich Waelsch and his colleagues. An earlier book with a similar title appeared in 1984 and, since then, there has been quite an upsurge of interest in the subject of transglutaminases (TGs). It attracts researchers from around the globe representing different subspecialities in biology, medicine and, not surprisingly, also in biomedical engineering mainly because of a need for bio-compatible polymers. Hundreds of participants attend special conferences held at frequent intervals; in recent years, a meeting in Ferrara, Italy in 2002, was followed by a symposium in Rome in 2003 and yet another one is scheduled for July 2005 as part of the FEBS-IUBMB meeting in Budapest, Hungary.

The Ca\textsuperscript{2+}-dependent TGs evolved from the papain family of proteases and, as such, operate with a Cys/His/Asp catalytic triad. However, the enzyme:substrate intermediate stabilizing function of a Gln residue in papain is replaced by the indole ring of a Trp in the active centers of all eukaryotic TGs, of which – excepting the catalytically inactive homologue of the band 4.2 protein – there are seven in the human genome.

The TG name, coined by Waelsch, is somewhat of a misnomer because these enzymes do not react with the free amino acid of glutamine; rather, they target the α-carbonylamide function in the side chain of Gln residues in protein substrates. Selection of a particular Gln depends less on the primary sequence surrounding it than on its location in the ternary structure of the protein. TGs seem to react best with Gln (acceptor) residues in unstructured flexible regions of proteins, often in the N and C terminal domains, but always in endo-positions.
Modification of the $\gamma$-carbonylamide group of the Gln residue depends on the nature of the electron pair donating nucleophile as the second reactant that might be water (causing hydrolysis), alcohol (esterification), a small amine (transamidation with a biogenic amine, mono-, di- or polyamine) or the $\varepsilon$-amino group of a Lys side chain of a protein. The simplified scheme below illustrates the outlines for some of the possible exchange reactions. Unlike papain, which is also known to promote transamidation, TGs display a high degree of specificity (saturable behavior) with regard to the amine donor substrate and also in the selection of Lys side chains in proteins with which they react; the enzyme-reactive Lys’s, too, are usually located in flexible domains of proteins.

Each reaction competes with all the others. Cross-linking of protein substrates can be blocked at the monomeric stage by the concomitant incorporation of extraneous amines (reaction 2) or short Gln peptides (reaction 3) into the participating protein partners. This proved to be an efficient way for probing reaction 1, often the focus of main interest in TG-catalyzed processes. Stabilization of human blood clots by thrombin-activated coagulation Factor XIII (FXIIIa) was the first such study where the small amines were shown to inhibit selectively only the covalent fusion of fibrin particles without interfering with their assembly into a clot network. By contrast, the same amines completely blocked the clotting of lobster blood and the formation of the copulation plug in rodents; the latter two are examples of nature’s simplest systems for the covalent polymerization of proteins where the relevant TG is sequestered into a compartment separate from its substrate. In fact, TGs may have been the earliest clotting enzymes in evolution; a TG isolated from the half billion year old species of Microciona (sea sponge) is efficient in clotting lobster plasma and any TG2 can stabilize the human fibrin clot, regardless whether it was derived from guinea pig liver or from human erythrocytes.
The introduction of Nε(γ-glutamyl)lysine side chain bridges by TGs – be it through stabilization of a pre-existing protein assembly or through the de novo direct polymerization of proteins – plays an important role in the organization of extracellular matrices. Similar, covalently linked membrane skeletal and cytoskeletal polymers are generated in cells when the latent TG becomes activated by the influx of Ca²⁺ (human red cells, keratinocytes) or by the action of some other signal (thrombin activation of platelets). Though a few intracellular TGs, just like Factor XIII, may require prior limited proteolytic processing, Ca²⁺ seems to be the universal trigger for the expression of TG enzyme activity. Much current research is focused on cross-linked polymers of cells with elevated Ca²⁺ contents, such as found in senescent cells, terminally differentiated cells and in cells undergoing pathological changes (Hb-Koln and sickle cell disease, cataract, neurodegenerative protein deposit diseases) and apoptosis. Reaction 1 in the scheme might be assumed to proceed with no change of free energy; nevertheless, this may be an essentially irreversible transition because the high molecular weight polymer probably forms a separate phase. Thus, efforts should be concentrated on preventing the TG-catalyzed maturation of pathological polymers with inhibitors rather than trying to reverse the process. TG inhibitory compounds might also be used to facilitate the lysis of blood thrombi, because stabilized clots are quite resistant to digestion by lytic enzymes.

One of the most interesting members of the family of TGs is TG2, originally discovered by the Waeclsh group. Even in this age of multifunctional proteins, one cannot help but marvel at the versatility of TG2. For example, in addition to its Ca²⁺-binding ability to assist the Cys/His/Asp/Trp catalytic unit in carrying out the enzymatic activities mentioned, this mid-size protein, along with several other TGs, binds GTP/GDP (the non-catalytic 4.2 band protein relative binds ATP). Nucleotide binding lowers the affinity for Ca²⁺ and inhibits the transamidating activity of the enzyme by cross-talk. Furthermore, TG2 can function as a G-protein (Gah) in signal transduction and binds to phospholipaseCβ. TG2 also forms a very tight complex with fibronectin and, at the interface of the cell with the extracellular matrix, this non-covalent unit influences cell migration.

The medical relevance of research on TGs is well documented. Perturbations in the activities of these enzymes are known to give rise to inherited hemorrhagic and skin diseases (Factor XIII deficiency and TG1 deficiency in lamellar ichthyosis). Much new information is expected from gene knockout experiments, such as the finding reported at the recent Ferrara meeting that TG2 plays a critical role in normal wound healing. There are also autoimmune conditions where a TG is the autoantigen. Inhibitory antibodies to Factor XIII may appear in the circulation and cause potentially fatal hemorrhage. In celiac disease, characterized by debilitating intestinal and systemic manifestations,
TG2 is the main target of autoantibodies, and symptoms in the related skin disease: dermatitis herpetiformis are caused by immune complex deposits of TG3.

This selection of articles illustrates the breadth of current research on transglutaminases, and it is hoped that the volume will stimulate further interest in this important field.

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References