predicted for an Ala for Trp (or Tyr) substitution. If a single Tyr is modified there is little effect on the catalytic activity measured with mM substrate (although for Y88A an increase in specific activity is seen). In the mammalian enzyme, one Trp is in the hydrophobic rim ridge and could be analogous to Trp47 in bacterial PI-PLC. Replacement of the rim Trp has little effect on binding of the protein to non-substrate containing vesicles (measured by RET of the protein to labeled PE incorporated into the vesicles). However, the activity is significantly reduced. These results (and analyses of other surface variants) are discussed in terms of the multi-domain structure of the mammalian PLC contributing to binding but with X-Y domain exhibiting similar conformational changes to the bacterial enzyme.

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Association Between Enzymes Modifies the Inhibition by Trehalose

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The crowding of the cell restricts the diffusion of solutes, provides specific binding sites for enzymes and promotes proteic interactions, allowing the metabolic channelling which favours a series of reactions in a pathway. (Srere PA. *Annu Rev Biochem*. 1987; 56:89-124) This process needs stable enzymatic interactions with low diffusion rate that give rise to multienzymatic complexes named metabolon.

The association modifies the kinetic properties and the relation between products and substrates, setting out that the enzymes form a complex among them or cellular structures. This suggests that the structural enzymatic organization exerts some control on the cellular metabolism.

Some factors can modify the equilibrium between the associated and soluble proteins, one of them being the viscosity promoted by the excess of compatible solutes like trehalose. (Kaushik J &, Bhat R. *J Biol Chem*. 2003; 278(29): 26458-65).

We decided to evaluate the effect of the trehalose over the glycolisis in yeast *Saccharomyces cerevisiae.* We had observed that in citoplasmic extracts the glycolisis is almost not affected by trehalose. But when we analyze some isolated enzymes we detected that some enzymes as aldolase and phosphoglycerate kinase (PGK) are not inhibited, while others like hexokinase and glyceraldehyde 3-phosphate dehydrogenase (GA3PDH) are inhibited. Enzymes of other pathways like glucose 6-phosphatase and glucose 6-phosphate dehydrogenase are also inhibited by the disaccharide.

To explain our results we did experiments with one sensible enzyme such as GA3PDH and a resistant PGK. This two may be associated and be part of the glycolitic metabolon. So in the coupled assay, the GA3PDH exhibit resistance to inhibition by trehalose. This suggests that the association stabilizes the sensible enzyme and it is probably specific because the combination of the GA3PDH with albumin, hexokinase and lactate dehydrogenase does not increase the resistance to trehalose.

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Kinetic Activity of the Intact 26S Proteasome in Mice Liver: Selective Regulation of Estrogen on the Core ß2 Subunit

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The 26S proteasome complex plays an essential role in intracellular protein degradation. The 26S complex contains one 20S proteolytic core and two 19S regulatory particles. We investigated the kinetic properties of the 26S proteasome and the potential regulation by estrogen in mice liver in control conditions (ovariectomized placebo treated) and after 10 days of estrogen treatment. Livers were homogenized with a low concentration of detergent to preserve proteasome integrity (mM): 50 Tris-HCl, 250 sucrose, 5 MgCl2, 2 ATP, 1 DTT, 0.5 EDTA, and 0.025% digitonin, pH 7.5. The assay buffer contained (mM): 50 Tris-HCl, 40 KCl, 5 MgCl2, 0.5 ATP, 1 DTT, pH 7.5. Activity was measured at 37 oC using three fluorescent substrates, ß1, caspase-like (Z-LLE-AMC), ß2, trypsin-like (Boc-LSTR-AMC), and ß5, chymotrypsin-like (Suc-LLVY-AMC). With all substrates, the proteolytic kinetics showed three phases: 1) a delay reflecting an initial rate-limiting process (binding of the peptide substrates to the 19S regulatory particles and the translocation to the proteolytic core), 2) a linear time-dependent proteolysis (degradation process in the 20S chamber), and 3) a saturation phase. Activity was measured as a function of substrate concentration (10-500µM) at constant total protein (ß1, ß2 100 µg; ß5, 50 µg). Increasing the substrate concentration did not affect the delay phase, while it increased the degradation rate and the saturation level. Increasing the protein concentration with constant substrate ß1, ß2 and ß5 50 µM) seemed to reduce the delay phase, while the linear activity and the saturation levels peaked at 100 µg protein. Estrogen treatment selectively stimulated proteolytic activity of the ß2 subunit trypsin-like activity. We conclude that proteasome activity has at least three sequential states with selective modulation by hormones of the proteolytic activity.

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Characterization of the Calcium Binding Domain of NADPH Oxidase 5 (NOX5)

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Superoxide generated by non-phagocyte NADPH oxidases (NOXs), such as NOX5, is of growing importance for vascular physiology and pathology. NOX5 enzyme consists of a transmembrane heme domain that is linked to a flavoprotein domain that contains FAD and binds NADPH. It appears to be regulated by self-contained Ca^{2+} binding domains (CaBD), which contains four EF-hands motifs. Previously we demonstrated that this calcium binding gates the heme reduction in NOX5, possibly thorough the CaBD-flavorprotein interaction. To better understand its structure and function, here we characterized the metal binding properties of the recombinant CaBD by fluorescent spectroscopy. Our data revealed that CaBD binds to Ca^{2+}, Mg^{2+}, terbium (Tb^{3+}) in the range of µM to mM. The data are further supported by the studies using the site-directed labeled CaBD. The rate of calcium association was too fast to be determined by a stopped-flow device, but the dissociation rate constant was determined to be 5 s^{-1} at 20 °C. The ANS titration and Stern-Volmer plots suggested that there was a significant conformational change upon the metal bindings. Interestingly, the spectra of circular dichorism indicated otherwise no change on the context of its secondary structure. However, this conformational change can be observed using the Surface Plasmon Resonance with the CaBD immobilized in the sensor chip. Because our data and other studies suggest there are two different types of calcium bindings in CaBD, currently we are performing the similar studies using N- and C-terminal halves of CaBD (aa 1-78 and 79-184), and mutants. We also are investigating the metal bindings in the CaBD of Dual Oxidase (DUOX), in which its hydrogen peroxide activity is controlled by calcium binding.

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Enzymatic Activity and Monolayer Binding of a Truncated Form of Lecithin Retinol Acyltransferase

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Lecithin retinol acyltransferase (LRAT) is a 230 amino acids membrane-associated protein. It has two enzymatic activities: first, it catalyzes hydrolysis of the sn-1 acyl chain of phospholipids and then transfers this acyl group to all-trans retinol to generate all-trans retinyl esters. This reaction is essential in the vertebrate visual cycle. The present study was performed to study the enzymatic activity of a truncated form of LRAT (tLRAT), where transmembrane domains have been removed. tLRAT extends from residues 31 to 196. It has been previously determined that the deleted domains of tLRAT do not contain residues known to be required for catalysis. tLRAT has been produced in *E. coli* and purified using affinity chromatography. Its enzymatic activity was studied using the short-chain diheptanoyl phosphatidylcholine (DHPC), which behaves like a mild detergent. The low critical micellar concentrations of DHPC allows to solubilize tLRAT and retinol. The maximal enzymatic activity of tLRAT is approximately 900 mol of ester/min • mol of protein. This value is more than 20 000 times higher than the largest enzyme activity reported in the literature. This huge difference can be explained by the use of a solution where DHPC serves both as a substrate as well as to solubililize the second substrate which highly favors the hydrolytic activity of tLRAT. Moreover, the injection tLRAT into the subphase of a phospholipid monolayer at different initial surface pressures allowed to determine the maximum insertion pressure (MIP) of tLRAT. A similar MIP of 38 mN/m has been obtained for dioleoyl phosphatidylcholine, ethanolamine and serine which is much higher than the lateral pressure of membranes. It can thus be postulated that tLRAT strongly binds membranes in the absence of its putative N- and C-terminal transmembrane domains.

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The Completion of chemo-mechanical coupling scheme of F_1-atpase; The Determination of the timing of Pi-release

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F_1-ATPase ($\alpha_3\beta_3\gamma$) is a rotary motor protein, which makes 120° step rotation upon one ATP hydrolysis. Extensive studies on F_1-ATPase revealed that each of three β-subunits, which has the catalytic site, follows the same reaction pathway of ATP hydrolysis, but they are always in a reaction phase differing by ± 120° from each other. When we focus on one β-subunit, the β binds ATP at a particular binding angle. After the γ rotates 200°, the β cleavages the bound ATP into ADP and Pi. The produced ADP is released from the β after further 40° rotation, at +240° from the ATP-binding angle. Then, when the γ makes