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ダイジェスト

JB COMMENTARY

Towards the in vivo identification of protein–protein interactions

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Protein–protein interactions (PPIs) play crucial roles in biological processes. The conventional methods based on affinity purification of a protein of interest (POI) have been widely used to identify unknown PPIs. Recently, proximity-dependent biotin identification (BioID) has been used increasingly to investigate PPIs. BioID utilizes the proximity-dependent biotinylation, in the presence of biotin, of endogenous proteins that are located within a certain distance of POI-fused biotin ligase, which enables us to reveal the more physiologically relevant PPIs *in vivo* compared to the conventional methods.

However, there is little information on an appropriate way to administer biotin *in vivo*. Recent studies reported some biotin supplementations for *in vivo* BioID. In this commentary, we review the BioID technique as a tool to examine PPIs, and we introduce a potential method to achieve efficient proximity labelling for *in vivo* BioID.

Keywords: cell death, chromo some dynamics, on gression index, micro tubu ledynamics, mitotic index

JB REVIEW

Lysine Acetyltransferases (KATs) in Disguise: Diseases Implications

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Acetylation is one of the key post-translational protein modifications catalysed by the protein lysine acetyltransferases (KATs). KATs catalyse the transfer of acetyl groups to the epsilon-amino groups of lysine residues in histones and non-histone proteins. Because of its wide range of target proteins, KATs regulate many biological processes, and their aberrant activities may underlie several human diseases, including cancer, asthma, Chronic Obstructive Pulmonary Disease (COPD), and neurological disorders. Unlike most of the histone modifying enzymes, such as lysine methyltransferases, KATs do not possess any conserved domain like SET domain of lysine methyltransferases. However, almost all the major families of KATs are found to be transcriptional coactivators or adaptor proteins, with defined catalytic domains, called canonical KATs. Over the past two decades, a few proteins have been discovered to possess intrinsic KAT activity but are not classical coactivators. We would like to categorize them as non-canonical KATs (NC-KATs). These NC-KATs include general transcription factors TAFII250, mammalian TFIIIC complex, and mitochondrial protein GCN5L1, etc. This review focuses on our understanding, as well as controversies regarding non-canonical KATs, where we compare the structural and functional similarities and dissimilarities of non-canonical KATs with the canonical KATs. This review also highlights the potential role of NC-KATs in health and diseases.

Keywords: non-canonical KATs, neurological disorders, Lysine acetyltransferases, cancer, bromodomain, Acetyl-CoA

REGULAR PAPER
BIOCHEMISTRY
Biochemistry General

Transcriptomic characterization of Lonrf1 at the single-cell level under pathophysiological conditions

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The LONRF family of proteins consists of three isozymes, LONRF1–3, which harbors RING (really interesting new gene) domain and Lon substrate binding domain. We have recently identified LONRF2 as a protein quality control ubiquitin ligase that acts predominantly in neurons. LONRF2 selectively ubiquitylates misfolded or damaged proteins for degradation. LONRF2$^{-/-}$ mice exhibit late-onset neurological deficits. However, the physiological implications of other LONRF isozymes remain unclear. Here, we

an alysed Lonrf 1 expression and transcriptomics at the single-cell level under normal and pathological conditions. We found that Lonrf1 was ubiquitously expressed in different tissues. Its expression in LSEC and Kupffer cells increased with age in the liver. Lonrf1^{high} Kupffer cells showed activation of regulatory pathways of peptidase activity. In normal and NASH (nonalcoholic steatohepatitis) liver, Lonrf1^{high} LSECs showed activation of NF-kB and p53 pathways and suppression of IFNa, IFNg and proteasome signalling independent of p16 expression. During wound healing, Lonrf1^{high}/p16^{low} fibroblasts showed activation of cell growth and suppression of TGFb and BMP (bone morphogenetic protein) signalling, whereas Lonrf1^{high}/p16^{high} fibroblasts showed activation of WNT (wingless and Int-1) signalling. These results suggest that although Lonrf1 does not seem to be associated with senescence induction and phenotypes, LONRF1 may play a key role in linking oxidative damage responses and tissue remodelling during wound healing in different modes in senescent and nonsenescent cells.

Keywords: regulation gene, proteolytic enzyme, mapping gene, expression gene, ageing diseases

Protein Structure

The cofactors and domains of a staphylococcal capsule-producing enzyme preserve its structure, stability, shape and dimerization ability

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CapF, a staphylococcal capsule-producing enzyme, binds Zn^{2+} ion and NADPH using its C-terminal domain (CTD) and N-terminal domain (NTD), respectively. To elucidate the roles of cofactors and domains, we have systematically investigated the related recombinant proteins, rCapF, rCTD, recombinant NTD (rNTD) and the Zn^{2+}-free rCapF/rCTD, Apo-rCapF/Apo-rCTD. The results show that the secondary structure, tertiary structure, shape and surface hydrophobicity of Apo-rCapF and Apo-rCTD are different from those of rCapF and rCTD. The removal of Zn^{2+} made rCapF thermo-sensitive, whereas both rCTD and Apo-rCTD are thermo-resistant proteins. Further, Apo-rCapF and rCapF existed as the dimers, whereas rCTD and Apo-rCTD formed a mixture of dimers and tetramers in the aqueous solution. Zn^{2+} maintained the structure of NTD as well. The NADPH binding activity and Cys accessibility of rNTD, rCapF and Apo-rCapF were significantly different from each other. The binding of NADPH to the above three proteins freely occurred, liberated heat at 25°C and increased their diameters. In addition, the structure, stability, shape and oligomerization ability of rNTD, rCTD and rCapF little resembled each other. Collectively, the domains and cofactors of CapF contribute to preserving its conformation, stability, shape and dimerization ability.

Keywords: structure, stability, shape, roles of domains and co-factors, CapF

Abbreviations: CapF, a capsule-producing enzyme expressed by Staphylococcus aureus; rCapF, CapF with the C-terminal end linked polyhistidine tag; Apo-rCapF, rCapF harbouring no zinc metal ion (Zn^{2+}); CTD, C-terminal domain of CapF; rCTD, CTD with the C-terminal end linked polyhistidine tag; Apo-rCTD, rCTD harbouring no Zn^{2+}; NTD, N-terminal domain of CapF; rNTD, NTD with the C-terminal end linked polyhistidine tag

Glycobiology and Carbohydrate Biochemistry

Establishment of a novel 70K Mac-2 binding protein antibody through screening of fucosylation-related antibodies

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Mac-2 binding protein (Mac-2bp) is a serum glycoprotein that contains seven *N*-glycans, and Mac-2bp serum levels are increased in patients with several types of cancer and liver disease. Mac-2bp glycosylation isomer has been applied as a clinical biomarker of several diseases, including liver fibrosis. In the present study, we identified fucosylated Mac-2bp in the conditioned medium of cancer cells resistant to anticancer therapies using glycoproteomic analyses. Fucosylation is one of the most important types of glycosylation involved in carcinogenesis and cancer stemness. To establish a next-generation glycan antibody for fucosylated Mac-2bp, we used fucosylation-deficient HEK293T cells to prepare reference Mac-2bp antigens and performed antibody screening. Unexpectedly, the 19-8H mAb obtained with our screen recognized 70K Mac-2bp, which is C-terminus-truncated product, rather than specifically recognizing fucosylated Mac-2bp. We performed immunocytochemistry using our novel 19-8H mAb, which resulted in strong cell surface staining of anticancer drug-resistant cancer cells. Therefore, our novel 19-8H mAb represents a valuable tool for cancer biology research that can help elucidate the biological function of 70K Mac-2bp.

Keywords: pancreatic cancer, Mac-2 binding protein, glycan antibody, fucosylation, 70K Mac-2bp

Abbreviations: AAL, Aleuria aurantia lectin; Mac-2bp, Mac-2 binding protein; Mac-2bp OE, Mac-2bp over expression; 19-8H mAb, 19-8H monoclonal antibody; Mac-2bp pAb, Mac-2bp polyclonal antibody; CA19-9, carbohydrate antigen 19-9; GMDS, GDP-mannose 4,6-dehydratase; ELISA, enzyme-linked immunosorbent assay; Mac-2bp OE cells, Mac-2bp overexpressing HEK293T; Mac-2bp OE and GMDS KO cells, Mac-2bp overexpressing HEK293T with GMDS knockout cells

Biochemistry in Diseases and Aging

Age-related alterations in protein phosphatase 2A methylation levels in brains of cynomolgus monkeys: a pilot study

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The abnormal activity of PP2A, a dominant member of type 2A serine/threonine protein phosphatase, has been implicated in the development of Alzheimer's disease (AD) and dementia with Lewy bodies (DLB). PP2A is a holoenzyme, and protein methylation of the catalytic subunit, PP2Ac, alters the complex composition. A decrease in PP2Ac methylation levels has been reported in AD and DLB. Aging is the most common risk factor for AD and DLB, but the relationship between aging and PP2A has not been studied in detail. Cynomolgus monkey show increased phosphorylation levels of tau and α-synuclein with aging. In this study, we investigated the alterations in the PP2A activity regulation with aging in monkey brains from 2 to 43 years of age using fractionated proteins. We found that type 2A protein phosphatase activity decreased with aging in cytoplasmic and nuclear-soluble fractions. PP2Ac methylation level was decreased in cytoplasmic and sarkosyl-insoluble fractions. A principal component analysis using PP2Ac, demethylated PP2Ac and PP2A methylesterase PME-1 levels in cytoplasmic and nuclear-soluble fractions as attributes showed that aged monkeys were in the same cluster. Our results show that brain aging in cynomolgus monkeys is closely related to changes in PP2A methylation.

Keywords: protein phosphatase 2 (PP2A), protein methylation, brain, animal model, aging

Abbreviations: CIP2A, cancerous inhibitor of PP2A; LCMT-1, leucine carboxyl methyltransferase 1; PCA, principal component analysis; PME-1, protein phosphatase methylesterase 1; PP2A: protein phosphatase 2A; SET, SE translocation

BIOTECHNOLOGY

RNA Technology

Inhibition of SARS-CoV-2 nucleocapsid protein–RNA interaction by guanosine oligomeric RNA

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The interaction of the β-coronavirus severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) nucleocapsid (N) protein with genomic RNA is initiated by specific RNA regions and subsequently induces the formation of a continuous polymer with characteristic structural units for viral formation. We hypothesized that oligomeric RNAs, whose sequences are absent in the 29.9-kb genome sequence of SARS-CoV-2, might affect RNA–N protein interactions. We identified two such hexameric RNAs, In-1 (CCGGCG) and G6 (GGGGGG), and investigated their effects on the small filamentous/droplet-like structures (< a few μm) of N protein–genomic RNA formed by liquid–liquid phase separation. The small N protein structures were sequence-specifically enhanced by In-1, whereas G6 caused them to coalesce into large droplets. Moreover, we found that a guanosine 12-mer (G12, GGGGGGGGGGGG) expelled preexisting genomic RNA from the small N protein structures. The presence of G12 with the genomic RNA suppressed the formation of the small N protein structures, and alternatively apparently altered phase separation to induce the formation of large droplets with unclear phase boundaries. We showed that the N-terminal RNA-binding domain is required for the stability of the small N protein structures. Our results suggest that G12 may be a strong inhibitor of the RNA–N protein interaction.

Keywords: SARS-CoV-2, RNA＜Viruses, RNA Interactions＜Protein, N protein, liquid–liquid phase separation

Abbreviation: COVID-19, Coronavirus Disease-2019; CTD, C-terminal domain; DIC, differential interference contrast; EMSA, electrophoretic mobility shift assay, IDR, intrinsically disordered region; FITC, fluorescein isothiocyanate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, LLPS, liquid–liquid phase separation; NTD, N-terminal domain; N, nucleocapsid; PEG, polyethylene glycol; PNA, peptide nucleic acid; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; SARS, severe acute respiratory syndrome coronavirus; vRNP, viral ribonucleoprotein

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JB REVIEW

Novel regulatory mechanisms underlying angiogenesis during wound healing revealed by fluorescence-based live-imaging in zebrafish

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Angiogenesis is a dynamic morphogenetic process that refers to the growth of new blood vessels from the pre-existing vessels and is critical for tissue repair during wound healing. In adult normal tissues, quiescent endothelial cells and pericytes maintain vascular integrity, whereas angiogenesis is immediately induced upon tissue injury, thereby forming neovascular networks to maintain homeostasis. However, impaired angiogenesis results in development of chronic and non-healing wounds in various diseases such as diabetes and peripheral artery diseases. Zebrafish are a vertebrate model organism widely used for studying many medical and life science fields. Indeed, the molecular and cellular mechanisms underlying regulation of wound angiogenesis have recently been studied by performing fluorescence-based live-imaging of adult zebrafish. In this review, we describe how endothelial cells and pericytes establish neovascular networks during wound angiogenesis and also introduce a novel role of blood flow-driven intraluminal pressure in regulating angiogenesis during wound healing.

Keywords: zebrafish, wound angiogenesis, TOCA family of F-BAR proteins, pericytes, endothelial cells

Msp1-mediated proofreading mechanism for localization of tail-anchored membrane proteins

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Protein targeting to organelles has been thought to be a very precise process, and proteins that fail to localize correctly are rapidly degraded. Tail-anchored proteins are posttranslationally targeted to the endoplasmic reticulum membrane *via* guided entry of tail-anchored (TA) proteins pathway. However, these proteins can be mislocalized to the mitochondrial outer membrane. We found that the AAA-ATPase Msp1 on the mitochondrial outer membrane extracts mislocalized TA proteins to the cytosol, passing them to the guided entry of the TA proteins pathway to facilitate their transfer to the endoplasmic reticulum membrane. After the transfer to the endoplasmic reticulum, such TA proteins are directed to degradation if they are recognized by the quality control system on the endoplasmic reticulum. If not recognized, they are retargeted to their original destination along the secretory pathway. Thus, we have identified an intracellular proofreading system that corrects the localization of TA proteins.

Keywords: GET pathway, mislocalization, Msp1, proofreading, TA protein

REGULAR PAPER
BIOCHEMISTRY
Biochemistry General

Response to acute hyperglycemia and high fructose in cultured tenocytes

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High monosaccharide levels are intimately associated with diabetes and impact tendon cells through inflammation and impairment in metabolic homeostasis. Experiments were designed to understand the responses elicited by cultured tenocytes under monosaccharide stress induced by hyperglycemia and hyperfructosemia. We simulated hyperglycemia and hyperfructosemia *in vitro* by treating tenocytes with media containing sublethal concentrations of glucose and fructose, respectively. Exposure of tenocytes to high glucose and high fructose altered the levels of IL-1β, IL-2, IL-6, IL10 and IL-17A. AMPK expression was increased in high-glucose and decreased in high-fructose groups. High fructose increased the level of IRS-1 compared with the control. Increased mitochondrial superoxide levels and compromised mitochondrial membrane integrity were exhibited by both the groups. The findings from the network analysis revealed many altered genes that are related to pathways for enzyme-linked receptor protein signaling, positive regulation of metabolic processes, transmembrane receptor tyrosine kinase pathway, insulin receptor signaling and regulation of cytokine production. Overall, the data suggest that the tenocytes under high monosaccharide levels exhibit survival responses by altering the expression status of cytokines and metabolic mediators that are involved in the underlying pathogenesis of tendinopathy.

Keywords: Diabetic tendinopathy, hyperfructosemia, hyperglycemia, rotator cuff tendon injury, tenocytes

Lipid Biochemistry

Nicotinamide-*N*-methyltransferase regulates lipid metabolism via SAM and 1-methylnicotinamide in the AML12 hepatocyte cell line

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Nicotinamide-*N*-methyltransferase (NNMT) is an enzyme that consumes *S*-adenosyl-methionine (SAM) and nicotinamide (NAM) to produce *S*-adenosyl-homocysteine (SAH) and 1-methylnicotinamide (MNAM). How much NNMT contributes to the quantity regulation of these four metabolites depends on whether NNMT is a major consumer or producer of these metabolites, which varies among various cellular contexts. Yet, whether NNMT critically regulates these metabolites in the AML12 hepatocyte cell line has been unexplored. To address this, we knockdown *Nnmt* in AML12 cells and investigate the effects of *Nnmt* RNAi on metabolism and gene expression. We find that *Nnmt* RNAi accumulates SAM and SAH, whereas it reduces MNAM with NAM being unaltered. These results indicate that NNMT is a significant consumer of SAM and critical for MNAM production in this cell line. Moreover, transcriptome analyses reveal that altered SAM and MNAM homeostasis is accompanied by various detrimental molecular phenotypes, as exemplified by the down-regulations of lipogenic genes, such as *Srebf1*. Consistent with this, oil-red *O*-staining experiments demonstrate the decrease of total neutral lipids upon *Nnmt* RNAi. Treating *Nnmt* RNAi AML12 cells with cycloleucine, an inhibitor of SAM biogenesis suppresses SAM accumulation and rescues the decrease of neutral lipids. MNAM also shows activity to elevate neutral lipids. These results suggest that NNMT contributes to lipid metabolism by maintaining proper SAM and MNAM homeostasis. This study provides an additional example where NNMT plays a critical role in regulating SAM and MNAM metabolism.

Keywords: (SAM), 1-methylnicotinamide (MNAM), AML12 cells, lipogenesis metabolism, nicotinamide-*N*-methyltransferase, S-adenosyl-methionine

Enzymology

Effects of Ca^{2+} ions on the horseshoe crab coagulation cascade triggered by lipopolysaccharide

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The lipopolysaccharide (LPS)-triggered horseshoe crab coagulation cascade is composed of three protease zymogens, prochelicerase C (proC), prochelicerase B (proB) and the proclotting enzyme (proCE). In this study, we found that Ca^{2+} ions increase the production of the clotting enzyme as a result of a cascade reaction reconstituted by recombinant proteins of wild-type (WT) proC, WT proB and WT proCE. We divided the cascade into three stages: autocatalytic activation of WT proC on the surface of LPS into WT α-chelicerase C (Stage 1); activation of WT proB on the surface of LPS into WT chelicerase B by WT α-chelicerase C (Stage 2) and activation of WT proCE into WT CE by chelicerase B (Stage 3). Ca^{2+} ions enhanced the proteolytic activation in Stage 2, but not those in Stages 1 and 3. Moreover, we performed isothermal titration calorimetry to clarify the interaction of LPS or the recombinant zymogens with Ca^{2+} ions. LPS interacted with Ca^{2+} ions at an association constant of $K_a = 4.7 \times 10^4 M^{-1}$, but not with any of the recombinant zymogens. We concluded that LPS bound with Ca^{2+} ions facilitates the chain reaction of the cascade as a more efficient scaffold than LPS itself.

Keywords: serine protease, recombinant protein, lipopolysaccharide, horseshoe crab hemolymph coagulation, calcium ions

Abbreviations: AMC, 7-amino-4-methylcoumarin; Boc, t-butoxycarbonyl; CBB, Coomassie Brilliant Blue; CE, clotting enzyme; EDTA, ethylenediaminetetraacetic acid; GnTI, N-acetylglucosaminyltransferase I; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HSA, human serum albumin; ITC, isothermal titration calorimetry; LPS, lipopolysaccharide; MCA, 4-methylcoumaryl-7-amide; proB, prochelicerase B; proC, prochelicerase C; proCE, proclotting enzyme; proG, prochelicerase G; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; SEM, standard error of the mean; WT, wild-type

Metabolism and Bioenergetics

Putrescine Biosynthesis from Agmatine by Arginase (TtARG) in Thermus thermophilus

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In the three domains of life, three biosynthetic pathways are known for putrescine. The first route is conversion of ornithine to putrescine by ornithine decarboxylase (ODC: SpeC), the second route is the conversion of arginine to agmatine by arginine decarboxylase (ADC: SpeA), followed by the conversion of agmatine to putrescine by agmatine ureohydrolase (AUH: SpeB), and the third route is the conversion of agmatine to *N*-carbamoylputrescine by agmatine deiminase (agmatine iminohydrolase, AIH), followed by the conversion of *N*-carbamoylputrescine to putrescine by *N*-carbamoylputrescine amidohydrolase (NCPAH). An extreme thermophile, *Thermus thermophilus* produces putrescine, although this bacterium lacks homologs for putrescine synthesizing pathways, such as ODC, AUH, AIH and NCPAH. To identify genes involved in putrescine biosynthesis in *T. thermophilus*, putrescine biosynthesis was examined by disruption of a predicted gene for agmatinase (agmatine ureohydrolase), or by using purified enzyme. It was found that arginase (TTHA1496) showed an agmatinase activity utilizing agmatine as a substrate. These results indicate that this bacterium can use arginase for putrescine biosynthesis. Arginase is a major contributor to putrescine biosynthesis under physiological conditions. The presence of an alternative pathway for converting agmatine into putrescine is functionally important for polyamine metabolism supporting survival at extreme environments.

Keywords: agmatine, aminopropylagmatine ureohydrolase, arginase, polyamine metabolism, putrescine, Thermus thermophilus

MOLECULAR BIOLOGY

Replication and Recombination

Biochemical characterization of the RNA-binding and RNA–DNA strand exchange activities of the human RAD52 protein

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RAD52 is a single-stranded DNA (ssDNA) binding protein that functions in the repair of DNA double-strand breaks (DSBs) by promoting the annealing of complementary DNA strands. RAD52 may also play an important role in an RNA transcript-dependent type of DSB repair, in which it reportedly binds to RNA and mediates the RNA–DNA strand exchange reaction. However, the mechanistic details of these functions are still unclear. In the present study, we utilized the domain fragments of RAD52 to biochemically characterize the single-stranded RNA (ssRNA) binding and RNA–DNA strand exchange activities of RAD52. We found that the N-terminal half of RAD52 is primarily responsible for both activities. By contrast, significant differences were observed for the roles of the C-terminal half in RNA–DNA and DNA–DNA strand exchange reactions. The C-terminal fragment stimulated the inverse RNA–DNA strand exchange activity displayed by the N-terminal fragment in *trans*, whereas the *trans* stimulatory effect by the C-terminal fragment was not observed in the inverse DNA–DNA or forward RNA–DNA strand exchange reactions. These results suggest the specific function of the C-terminal half of RAD52 in RNA-templated DSB repair.

Keywords: RNA, protein-nucleic acid interaction, intrinsically disordered region, homologous recombination, DNA repair

CELL

Biomembranes, Organelles and Protein Sorting

Inner nuclear membrane proteins Lem2 and Bqt4 interact with different lipid synthesis enzymes in fission yeast

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The nuclear envelope (NE) is a double-membrane structure consisting of inner and outer membranes that spatially separate the nucleus from the cytoplasm, and its function is critical for cellular functions such as genome maintenance. In the fission yeast, *Schizosaccharomyces pombe*, the inner nuclear membrane proteins, Lem2 and Bqt4, play pivotal roles in maintaining the NE structure. We previously found that the double deletion of *lem2*$^+$ and *bqt4*$^+$ causes a synthetic lethal defect associated with severe NE rupture, and overexpression of Elo2, a solo very-long-chain fatty acid elongase, suppresses this defect by restoring the NE. However, the molecular basis of this restoration remains elusive. To address this, we identified Lem2- and Bqt4-binding proteins via immunoprecipitation and mass spectrometry in this study. Forty-five and 23 proteins were identified as Lem2- and Bqt4-binding proteins, respectively. Although these binding proteins partially overlapped, Lem2 and Bqt4 interacted with different types of lipid metabolic enzymes: Cho2, Ole1 and Erg11 for Lem2 and Cwh43 for Bqt4. These enzymes are known to be

involved in various lipid synthesis processes, suggesting that Lem2 and Bqt4 may contribute to the regulation of lipid synthesis by binding to these enzymes.

Keywords: Bqt4, Lem2, nuclear envelope, proteome analysis, Schizosaccharomyces pombe

BIOTECHNOLOGY

Drug Delivery Systems

Thermodynamic stability of human lipocalin-type prostaglandin D synthase under various pH conditions

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Lipocalin-type prostaglandin D synthase (L-PGDS) binds various hydrophobic small molecules. Since we aim to use human L-PGDS as a carrier in a drug delivery system (DDS) for poorly water-soluble drugs, quality control of the protein is indispensable. In this study, we investigated the thermodynamic stability of human L-PGDS under various pH conditions. Differential scanning calorimetry revealed that the thermal unfolding of L-PGDS was an almost-reversible two-state transition between the native and unfolded states over the pH range from 2.5 to 7.4. The linear relationship of $\Delta H(T_m)$ to T_m in this pH range gave a heat capacity change (ΔC_p) of 4.76 kJ/(K·mol), which was small compared to those commonly found in globular proteins. The temperature-dependent free energy of unfolding, $\Delta G(T)$, specified by T_m, $\Delta H(T_m)$ and ΔC_p, showed a pH dependence with the highest value at pH 7.4 closest to the isoelectric point of 8.3. The small value of C_p resulted in a large value of $\Delta G(T)$, which contributed to the stability of the protein. Taken together, these results demonstrated that human L-PGDS is sufficiently thermostable for storage and practical use and can be useful as a delivery vehicle of protein-based DDS.

Keywords: two-state transition, thermodynamic stability, human L-PGDS, heat capacity changes, drug delivery system