The cover picture shows how the carbon–carbon bond formation in a protein has been achieved by a combined approach of organic synthesis and genetic engineering. Palladium-catalyzed reactions have been used in the synthesis of a wide variety of organic molecules. Among them, the Mizoroki–Heck reaction allows alkenylation of an aryl iodide to be performed under mild conditions. In the article by S. Fuku- zawa, K. Tachibana, S. Yokoyama, et al. on p. 134 ff, it is demonstrated how fragile biomolecules like proteins can be substrates for palladium-catalyzed reactions.

MINIREVIEWS

J. M. Bujnicki*

19 – 27

Protein-Structure Prediction by Recombination of Fragments

Convergence of protein folding and protein evolution. Recent developments in both template-based and template-free protein-structure modeling are summarized, and the available methods for protein-structure prediction by recombination of fragments are compared. Convergence between the “protein folding” and “protein evolution” schools of thought is postulated.

COMMUNICATIONS

J. A. Kritzer, R. Zutshi, M. Cheah, F. A. Ran, R. Webman, T. M. Wongjirad, A. Schepartz*

29 – 31

Miniature Protein Inhibitors of the p53–hDM2 Interaction

Small but perfectly formed. A library of miniature protein variants was constructed that presented the minimal recognition epitope of the human double-minute 2 oncoprotein (hDM2), which was derived from the activation domain of p53 (p53AD). This library was optimized (see scheme) to yield several miniature proteins with robust folds and nanomolar affinity for hDM2. The inhibitory activities of these miniature proteins correlated with the stability of the protein fold. This emphasizes the benefit of presenting the p53AD epitope on a miniature protein scaffold.
**Not inhibited.** We have successfully synthesized and tested a panel of activity-based small-molecule probes that target different classes of proteases on the basis of their enzymatic activities and substrate specificities rather than their inhibition. We have demonstrated that these probes are useful for generating unique substrate fingerprint profiles of proteases in gel-based proteomic experiments. Preliminary results indicate that they might be equally amenable for microarray-based enzyme profiling experiments.

**Part of the queue.** Biosynthesis of the cytostatic polyketide aureothin (1) in Streptomyces thioluteus was found to involve two tailoring steps (see scheme). Mutational biosynthesis of nor-deoxy-aureothin (α-2) and a hydroxylated derivative (3), together with biotransformation experiments revealed a well-defined order for the polyketide-tailoring steps. Regioselective γ-pyrone methylation was found to be the penultimate biosynthetic step prior to furan-ring formation.

**A different kind of breakdown.** The structure of At-NCC-3, a polar nonfluorescent chlorophyll catabolite (NCC) from the model plant Arabidopsis thaliana, deviates curiously from the known chlorophyll-breakdown pathway in higher plants: At-NCC-3 carries a hydroxymethyl group instead of a methyl group at C(7), a much discussed, specific structural link of the known NCCs with chlorophyll(ide) a.

**The characteristic time** for the contact formation between two helical segments, a fundamental step in the protein-folding pathway, has been determined by time-resolved optical spectroscopies on a model bioactive peptide.
J. López de la Osa, C. González,* R. Gargallo, M. Rueda, E. Cubero, M. Orozco,* A. Aviñó, R. Eritja*

Destabilization of Quadruplex DNA by 8-Aminoguanine

Getting rid of unwanted quadruplexes. Oligonucleotides carrying 8-amino-2′-deoxyguanosine form very stable triple-helical structures. In this paper, we show that 8-amino-2′-deoxyguanosines destabilize quadruplex DNA structures. This quadruplex-destabilization effect is very convenient for the design of triple-forming oligonucleotides, making 8-aminopurines superior to other purine derivatives.

O. Khersonsky, D. S. Tawfik*

Chromogenic and Fluorogenic Assays for the Lactonase Activity of Serum Paraoxonases

5-Thioalkyl butyrolactones (1) are shown to be useful chromogenic/fluorogenic probes for determining lactonase activities, in particular of serum paraoxonase (PON1). These novel probes are suitable for measuring lactonase activity in complex biological samples, including sera, intact cells and cell lysates.

F. Cachoux, T. Isarno, M. Wartmann, K.-H. Altmann*

Total Synthesis and Biological Assessment of Benzimidazole-Based Analogues of Epothilone A: Ambivalent Effects on Cancer Cell Growth Inhibition

Benzimidazole-based analogues of cis- and trans-Epo A, 2 and 3, have been prepared through stereoselective total synthesis. Both compounds are highly potent antiproliferative agents, but the effects of side-chain replacement on cellular activity are ambivalent. While significantly enhanced potency is observed against a drug-sensitive human cancer cell line, 2 and 3 more susceptible to P-gp-mediated drug efflux than Epo A or trans-Epo A.

FULL PAPERS

P. Berthault,* G. Huber, P. T. Ha, L. Dubois, H. Desvaux, E. Guittet

Study of the Hydrophobic Cavity of β-Cryptogein through Laser-Polarized Xenon NMR Spectroscopy

Magnetization transfer. The cavity of β-cryptogein has been investigated by laser-polarized xenon NMR. Whereas 1H and 13C experiments do not show significant chemical shift variation with increasing xenon pressure, the use of polarization transfer from the dissolved noble gas to protons undoubtedly reveals an interaction site.
Optimization of Xenon Biosensors for Detection of Protein Interactions

Possible lead compound in antidiabetes research: The dipeptide H-Trp-Glu-OH (G3335) was discovered to be a novel PPARγ antagonist, as confirmed by surface plasmon resonance and use of yeast two-hybrid systems and mammalian transactivation assays. The significance of the Cys285 residue in PPARγ for PPARγ-LBD–G3335 interaction was investigated by homology modeling, molecular docking and point-mutation analyses.

Expanding the repertoire. The unsaturated analogue SS-E-Ile (see scheme) was used instead of isoleucine during recombinant-protein expression. The analogue was incorporated into mouse dihydrofolate reductase with an efficiency of up to 72%. This strategy gives protein chemists access to the versatile alkene functionality for protein modification.

Traffic control. Avidin conjugates and biotinylated peptides that carry an O-phosphorylated or O-linked N-acetylglucosamine (O-GlcNAc)-modified serine next to the nuclear-localisation sequence (NLS) of the viral Jun protein were synthesised. Introduction of fluorescently labelled biotinylated NLS peptides into NIH/3T3 (see figure) and MDCK cells revealed that the unmodified form is rapidly imported into the nucleus, although either a phosphate or O-GlcNAc modification prevents this.

Influence of Serine O-Glycosylation or O-Phosphorylation Close to the vJun Nuclear Localisation Sequence on Nuclear Import

ChemBioChem 2006, 7, 6–14 © 2006 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim www.chembiochem.org
A. R. Feldman, E. K. Y. Leung, A. J. Bennet, D. Sen*  
98–105  
The RNA-Cleaving Bipartite DNAzyme Is a Distinctive Metalloenzyme  

Go your own way. The bipartite DNAzyme is an efficient in vitro-selected catalytic DNA for general RNA cleavage. Kinetic analysis suggests that it is an obligate metalloenzyme that makes use of two metal cations in a mechanism that is distinct from those of other small ribozymes and DNAzymes.

K. Kastl, M. Menke, E. Lüthgens, S. Faiß, V. Gerke, A. Janshoff, C. Steinem*  
106–115  
Partially Reversible Adsorption of Annexin A1 on POPC/POPS Bilayers Investigated by QCM Measurements, SFM, and DMC Simulations  

An on/off relationship: The reversibility of annexin A1 binding to lipid bilayers containing phosphatidylserine (k_on, k_off, and k_irr are the rate constants for reversible adsorption, desorption, and irreversible adsorption, respectively) as a function of [Ca^{2+}] was elucidated by a combination of quartz crystal microbalance measurements and dynamic Monte Carlo simulations. Not only are the reversibility and the kinetic parameters of protein binding influenced by the calcium ions but also the structure of the membrane itself.

S. J. Williams, O. Hekmat, S. G. Withers*  
116–124  
Synthesis and Testing of Mechanism-Based Protein-Profiling Probes for Retaining Endo-glycosidases  

Illuminating retaining endo-glycosidases. Mechanism-based probes were synthesized through conjugation of a biochemical tag, biotin (see picture), to a specific and efficient 2-deoxy-2-fluoro-sugar inactivator of retaining glycosidases. Experiments with pure glycosidases and the secreted proteome of Cellulomonas fimi illustrated the power of this approach for targeting and identification of retaining endo-xylanases in complex proteomes.

A. Kupan, A. Saulière, S. Broussy, C. Seguy, G. Pratviel,* B. Meunier  
125–133  
Guanine Oxidation by Electron Transfer: One-versus Two-Electron Oxidation Mechanism  

Radical leads to lesions: The fate of a guanine radical cation, the product of one-electron oxidation of guanine in DNA, was shown to follow a major pathway, deprotonation followed by trapping by O_3 (see scheme). This leads to imidazolone (Iz) as the major guanine lesion. A minor two-electron oxidation pathway was evidenced by the formation of guanine lesions like 8-oxo-7,8-dihydroguanine and tris(hydroxymethyl)-aminomethane (Tris) adducts.
Site-specific functionalization. A new carbon–carbon bond in a protein was formed by means of organopalladium chemistry. A vinylated biotin was coupled with a 4-iodo-L-phenylalanine residue on the Ras protein by a Mizoroki–Heck reaction. The biotinylated Ras showed binding activity for its downstream target with no signs of decomposition.

Lack of lactone. The naturally occurring lipopolysaccharide (LPS) from *Rhizobium* sin-1 can antagonize the production of the tumor necrosis factor TNF-α by enteric LPS. As it is not known whether a 2-aminogluconolactone or 2-aminogluconate form is responsible for this property, compound 4, which contains a methyl ether at the C-5 hydroxy group preventing lactonization, was prepared and its proinflammatory properties were determined.

Suicidal selection: Mutants of *Bacillus subtilis* lipase A were displayed on bacteriophages. Dual selection with the aid of (S)-(−) and (R)-(−)-IPG stereoisomers coupled to phosphonate suicide inhibitors was used to isolate variants with inverted enantioselectivity. The 3D structures of the inhibitor–lipase complexes were determined, providing structural insight into the mechanism of enantioselectivity of the enzyme.

A putative prenyltransferase gene from *Aspergillus fumigatus* AF293—*fgaPT1*—was cloned and overexpressed in *Escherichia coli*. The His₆-fusion *FgaPT1* was purified to near homogeneity and characterized biochemically. This enzyme was found to convert fumigaclavine A (1) into fumigaclavine C (2) by attaching a dimethylallyl moiety to C-2 of the indole nucleus in a “reverse” manner. The biochemical properties of *FgaPT1* have been investigated in this study.

K. Kodama, S. Fukuzawa,* H. Nakayama, T. Kigawa, K. Sakamoto, T. Yabuki, N. Matsuda, M. Shirouzu, K. Takio, K. Tachibana,* S. Yokoyama*

134 – 139
Regioselective Carbon–Carbon Bond Formation in Proteins with Palladium Catalysis; New Protein Chemistry by Organometallic Chemistry

H.-S. Lee, M. A. Wolfert, Y. Zhang, G.-J. Boons*

140 – 148
The 2-Aminogluconate Isomer of *Rhizobium* sin-1 Lipid A Can Antagonize TNF-α Production Induced by Enteric LPS

M. J. Dröge, Y. L. Boersma, G. van Pouderoyen, T. E. Vrenken, C. J. Rüggeberg, M. T. Reetz, B. W. Dijkstra, W. J. Quax*

149 – 157
Directed Evolution of *Bacillus subtilis* Lipase A by Use of Enantiomeric Phosphonate Inhibitors: Crystal Structures and Phage Display Selection

I. A. Unsöld, S.-M. Li*

158 – 164
Reverse Prenyltransferase in the Biosynthesis of Fumigaclavine C in *Aspergillus fumigatus*: Gene Expression, Purification, and Characterization of Fumigaclavine C Synthase FGAPT1
Five against five: A novel 5-membered iminocyclitol derivative (1) was found to be a potent and selective inhibitor of the glycoprotein-processing α-glucosidase with a $K_i$ value of 53 nM. It was further developed into antiviral agents such as 2 (against Japanese encephalitis virus, dengue virus serotype 2, human SARS coronavirus, and human β-hexosaminidase ($K_i = 2.6$ nM), a new target for development of osteoarthritis therapeutics.

Controlling the control. Calcium is the single most important intracellular second messenger; it controls our locomotion, heartbeat, and memory storage. Cells use a basic “tool kit” to give Ca$^{2+}$ such multifarious functions. Subcellular control of Ca$^{2+}$ concentration by inositol-1,4,5-trisphosphate (IP$_3$) is a key component of this kit. Here we introduce a new chemical probe that permits focal, two-photon uncaging of IP$_3$ in living cells (see scheme).

Trading places. Synthetic analogues of the natural aspartic-proteinase inhibitor pepstatin A and its methyl ester have been prepared. Both have two Tfm groups that replace the native isobutyls and are low-nanomolar inhibitors of plasmepsin II (PM II) with good selectivity against human cathepsins D and E. The structural effects and role of the Tfm group were investigated by solving the crystal structures of PM II bound to the bis-Tfm pepstatin A analogues.

NMR studies show that in the flavin-binding BLUF domain of AppA, a blue-light photoreceptor protein, considerable disorder is observed for residues near the flavin chromophore due to conformational exchange both in the dark and in the light-induced signaling state of AppA. Light-induced structural changes in a patch of surface residues provide a structural link between light absorption and signal-transduction events that inhibit the antirepressor function of AppA.
A simple and reliable approach for the generation of protein microarrays based on self-immobilizing fusion proteins is presented. Important features of the approach are the selectivity of the immobilization and the opportunity both to label and to immobilize the employed fusion proteins, which allows for the direct screening for protein–protein interactions. In addition, these protein microarrays can be used for the characterization of small molecule–protein interactions or post-translational modifications.
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