PREFACE

This book presents and discusses a variety of research done in the field of biochemistry, with a particular focus on ligands, polymers and amino acids. Topics discussed include G-Quadruplex Ligands and the evaluation of a synthetic biomimetic ligand used for the purification of therapeutic proteins; phytoestrogens biochemical aspects and biological activities; increase in amino acids and ammonia during liquefaction of semen; the structure of inhibitor polymers and prebiosis with fermentable carbohydrates in pig nutrition.

Chapter 1 - G-quadruplex represents a group of unusual DNA secondary structures, based on Hoogsteen G-G pairing hydrophobic planar rings of four guanines. Many studies have reported that G-quadruplex structures appear to be involved in many important biological processes, such as DNA replication, gene expression and recombination, as well as cell transformation. The G-quadruplex has been shown to be specifically important in the structure of G-rich single-strand DNA at the end of telomeres. The presence of these structures in vivo has been demonstrated in a number of biological systems, ranging form ciliates to humans. For these reasons, in the last few years, the research of specific G-quadruplex ligands as potential drugs has gained major attention, in particular as telomeres targeting agents and telomerase inhibitors and thus potential anticancer drugs.

Many eminent scientists have been working in this exciting field, as demonstrated by the success of the First International Meeting on Quadruplex DNA, held in Louisville (KY, USA) in April 2007§. The specificity of the interactions between the studied ligands and different DNA structures (both duplex and quadruplex) is surely a main topic in this area, since it determines the different biological effects of these compounds. Several approaches have been used to explore this subject, and they will be briefly summarized in this chapter. Nevertheless, particular attention should be paid when comparing biological data from different labs on telomerase inhibition by these compounds. In fact, these data, which are very important from a pharmaceutical and medicinal chemistry point of view, are affected by many factors, such as the type of cells used to extract telomerase, the presence of proteins other than telomerase in the cell extract, the amount of cell extract and DNA substrates used, as well as other features that have not been fully standardized yet.

Chapter 2 - The successful commercialization of high-purity proteins and enzymes parallels the development and improvement of downstream processing technology. Biomimetic ligands find wide application in protein separation and purification, being the most promising affinity ligands of large-scale potential. In the present paper we evaluate the ability of the biomimetic ligand 4-amino-phenyl-oxanilic acid coupled to Sepharose CL-6B

via 1,3,5-trichloro-2,4,6-triazine to bind and purify human monoclonal anti-HIV antibody 2F5 (mAb 2F5) from spiked maize extracts and recombinant influenza virus neuraminidase (NA) expressed in insect cells. Under selected conditions the affinity adsorbent exhibited high selectivity and purifying ability for mAb 2F5 and NA.

Chapter 3 - Phytoestrogens are natural compounds found in plants that possess estrogen-like activity. The major classes of phytoestrogens are isoflavones, lignans and coumestans. Isoflavones are structurally similar to mammalian estrogen and are found in large amounts in soybean and soy products. Lignans are found in flaxseed, whole grain cereals, teas and some vegetables, and coumestans are mainly present in clover and alfalfa sprouts. The major isoflavones are genistein, daidzein and glycitein. These compounds are hydrolysis products of glycosides in plants, termed genistin, daidzin and glycitin, which, after oral ingestion, are metabolized by intestinal flora. In lignans, the plant precursors are matairesinol and secoisolariciresinol, which are also metabolized by the intestinal microflora to enterolactone and enterodiol, respectively. Phytoestrogens have been largely used in the world and for a long time period, and it has been observed that the incidence of hormone-dependent diseases is reduced in countries, especially Asian countries, with a high dietary content of phytoestrogens. Animal models and clinical investigations have shown that phytoestrogens are protective in the prevention of hormone-dependent cancers, primarily breast and prostate cancers; cardiovascular disease; osteoporosis and to alleviate the symptoms of menopause. The estrogenic effects of the phytoestrogens are mediated through the estrogen receptors ERα and ERβ, which function as ligand-inducible transcription factors for genes involved in cell growth, proliferation, and differentiation. Many mechanisms of action have been identified for phytoestrogen prevention of diseases, including estrogenic/antiestrogenic activity, antiproliferation, induction of cell cycle arrest and apoptosis, antioxidation activity, induction of detoxification enzymes, regulation of the host immune system, and changes in cell signaling. Despite growing evidence of the health benefits of phytoestrogens, it is important to investigate further their mechanism of actions for a better understanding of the biological outcomes and possible adverse effects.

Chapter 4 - The metastable structure of a serpin a1-proteinase inhibitor (a1-PI) makes it prone to conformational changes as a result of certain mutations and under mild denaturing conditions. Polymers of several variants of a1-PI, e.g., the most clinically relevant Z variant, accumulate in the liver and are believed to be the underlying cause of the liver disease associated with a1-PI deficiency. Such polymers have also been found in the circulation and in the lung. The structure of these polymers, which is important for structure-based drug design, remains unknown. The historically first and generally accepted model of the a1-PI polymers, the loop-A sheet model, which assumes insertion of the reactive center loop (RCL) of one a1-PI molecule between the central strands of the A b-sheet of another molecule, has never been proven. In addition, this model is inconsistent with resonance energy transfer data obtained for heteropolymers formed from the Z and S variants. It is also difficult to envision how this model or even the more compact loop-A sheet model, deduced from fluorescence data obtained for the Z and S variant heteropolymers, can be compatible with electron microscopy (EM) images, which show apparently flexible polymer chains. Two other polymer models were deduced from the interactions seen between molecules in the crystals of two other serpins, antithrombin (AT) and plasminogen activator inhibitor-1 (PAI-1), which demonstrated the ability of the reactive center loop (RCL) to assume the b-strand conformation and to extend the C or A b-sheet in a neighboring molecule by formation of an

additional edge strand. This became the ground for the loop-C sheet and strand s7A models of the polymer, although the interactions seen in crystals appear not to be stable in solution and, thus, appear not to reflect interactions existing in a1-PI polymers, which actually are stable. The focus of this paper is to review the observations used to support the proposed models and to revisit the wealth of literature data in search for unexplained observations and possible new interpretations. I also put forward the hypothesis that a fusion of b-sheets, rather than insertion of the reactive center loop into a b-sheet, may underlie the polymerization process of a1-PI. This hypothesis is an expansion of the recent head-to-head model proposed based on the polymerization of the a1-PI disulfide-linked dimer.

Chapter 5 - Fresh normal ejaculate clots by action of about 0.05 IU/ml thrombin on a fibrinogen-like compound, and subsequently lyses by action of about 0.1 IU/ml plasmin and about 300 % (of blood plasma norm) carboxypeptidase. Carboxypeptidases sequentially liberate amino acids, that were quantified in the present work: the most abundant amino acids in fresh seminal plasma are serine (6.09 mM), threonine (4.61 mM), lysine (3.45 mM), tyrosine (3.18 mM), glycine (2.79 mM), and arginine (2.29 mM). The concentration of the amino acids approximately doubles within 45 min (37°C), with an exceptional 12fold increase of glutamic acid. When compared to blood plasma, fresh seminal plasma contains 226fold gamma-glutamyl-transferase (γ-GT), 40fold ammonia, 21fold creatinine, and 18fold glutamate dehydrogenase (GLDH). Ammonia (NH3), generated by glutamate dehydrogenase (GLDH), together with arginine or creatinine might protect sperm cells from female defence cells. Ammonia and natural guanidine derivatives might be physiologic immunsuppressors.

Chapter 6 - Evolutionary processes over time have formed a symbiotic relationship between a well diverse and stable microbiota and the animal host. Berg [1] and Gaskins [2] have pointed out that bacterial cells outnumber animal cells by a factor of 10 and have a profound influence on nutritional, physiological, immunological, and protective processes in the host.

Essentially, gastrointestinal tracts of animals are sterile at birth. After the first introduction to microflora during birth, the GI tract gets colonized by microbes from mother and the environment, until a stable, diverse and complex microbiota exists. The successful development, stability, and functionality of this very complex ecosystem is dependent on the microbial community, host physiology and the diet (nutrient source for the ecosystem). Any alterations in these three pillars of the GIT microbial ecosystem, lead to changes in microbial activity with consequences on host physiology and performance.

This chapter is focused on the effects of prebiotics, essentially fermentable carbohydrates in the diet, on the gut microbial community, gut health, physiology and performance of the animal, and the environment in and around a pig farm. Author further discusses the candidature of fermentable carbohydrates as an alternative to in-feed antibiotics in pig production. Some recommendations for further research directions are also discussed.

Chapter 7 - Mycotoxins are toxic or carcinogenic compounds produced by fungi, including Aspergilllus and Fusarium molds that colonize seeds of maize, cotton, peanuts or tree nuts. Insect herbivory of plant tissue often enhances mold infection. Transgenic maize expressing the Bacillus thuringiensis (Bt) toxin produce ears with lower mycotoxin levels when the target insect pest is effectively controlled. However, Bt toxins are generally species specific and therefore new transgenic strategies for broad insect control need to be developed. The vast diversity of plant secondary biochemicals reflects the strategy of non-motile plants to defend themselves from pathogens and animal herbivores. Research in recent years

revealed that many of these plant biochemicals are synthesized by a number of enzymes that are coordinately regulated at the gene level by transcription factors. Altering the expression of these transcription factors by genetic engineering opens the possibility that these secondary biochemicals may be synthesized in specific tissues to combat insect herbivory and reduce mycotoxin contamination. Alternatively, well studied secondary biochemical pathways may be modified or transferred from one plant species to another and tested for effective insect resistance. Transgenic manipulation of secondary metabolism must be carefully considered because redirection of plant energy reserves may hinder plant yield. The advent of genetic engineering and advances in plant biochemistry has opened up the exciting possibilities of utilizing the diversity of secondary biochemicals for protecting valuable crop commodities.

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