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NUTRITION, HEALTH AND FUNCTIONAL FOODS

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Characteristics of Nutrition in Japan

Nowadays we have an abundant supply of food in Japan, and we also import 5,800 million tons of food from abroad. Rather satiation than shortage of food is a big problem: 7.7 % of food at home is discarded and 23.9 % of dish is threw away as garbage at a wedding reception. Quality matters more than quantity in nutrition in our country.

The center of public attention in food is lipid, as it is directly related to the atherosclerosis and coronary heart disease. According to the Recommended Dietary Allowances for Japanese (6th edition, 2000), the percentage of calories from fat is 20 - 25 % for adult, and the (n-3)/(n-6) ratio is recommended as 1:4. The S/M/P ratio is desirable to be 3:4:3. National Nutrition Survey in 1999 revealed that the average intake is nearly the ideal level.

We eat fish and shellfish more than meat, so we take a fairly large amount (n-3) lipid as compared to European and North American countries. As already pointed out in "Seven Countries Study" by Keys and Kimura (1), the incidence of coronary death in Japan is low and the life span of Japanese is the longest in the world.

Under such circumstance, scientific evidence of nutrition is longed not to deviate from the ideal health condition.

Fatty Acid Synthesis in Our Body

We store energy as triacylglycerol and contain lipids as constituents of the plasma membrane. Signal transduction through the plasma membrane is the third but important function of lipids. Fatty acids are the common components of these lipids, and most of them are synthesized in our body itself by the enzyme named fatty acid synthase, using acetyl-CoA and malonyl-CoA as substrates and NADPH as a cofactor. Usually palmitic or stearic acid is the product of this process. Oleic acid is then produced from stearic acid by delta-9 desaturase.

Fatty acid synthase is a multifunctional enzyme, having seven kind of activities on a single peptide chain: acetyl transacylase, malonyl transacylase, β-ketoacyl synthase, β-ketoacyl reductase, β-hydroxyacyl dehydratase, enoyl reductase, and thioesterase. Fatty acid synthase catalyzes these sequential reactions, and after seven repetitions of these six reactions except thioesterase, palmitic acid is released from the enzyme by thioesterase reaction (Fig. 1). All the intermediates are covalently bound to the protein molecule during this process.

We purified this enzyme from the Harderian gland of guinea pig, and determined the quartery structure of the enzyme (2) (Fig. 2). The active enzyme is a dimer, consisted of two subunits of Mr=2.5 x 10^5. The negatively stained enzyme had an electron micrographic image of an ellipsoidal contour with

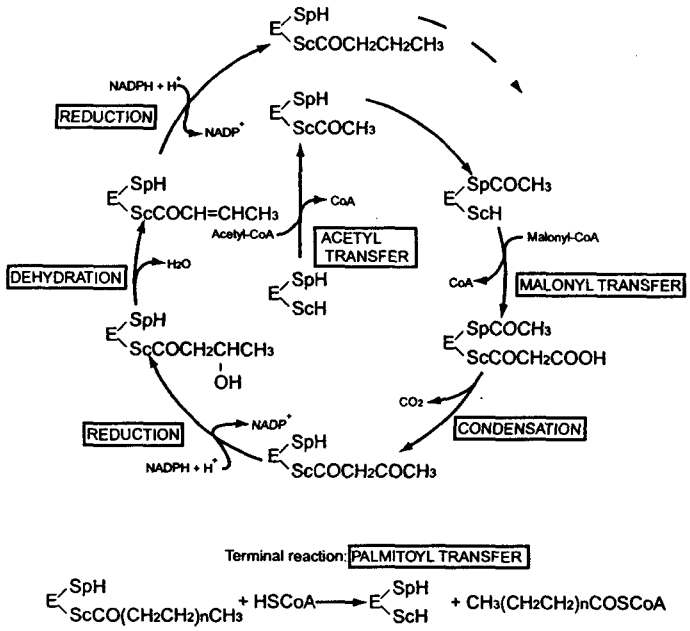


Fig. 1 Schematic representation of fatty acid synthesis.

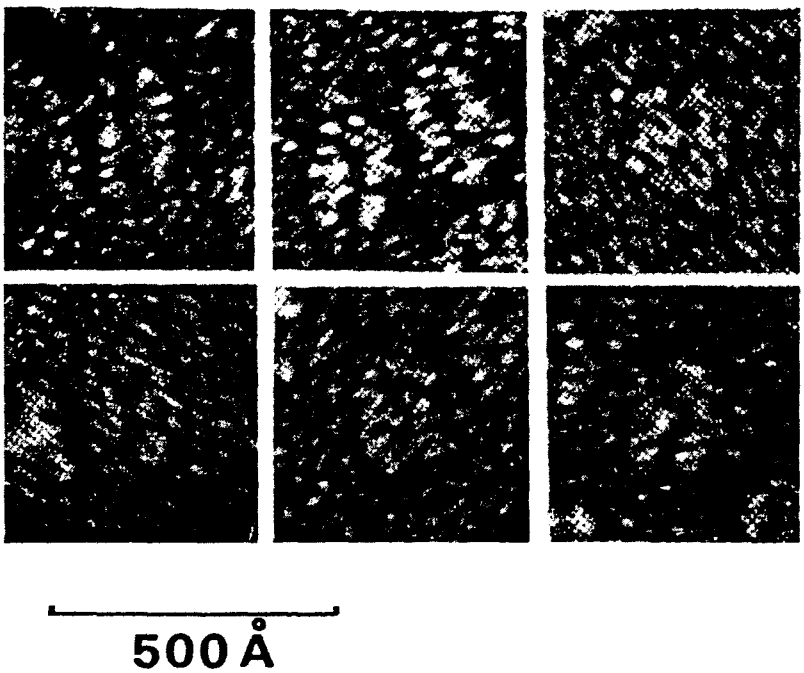
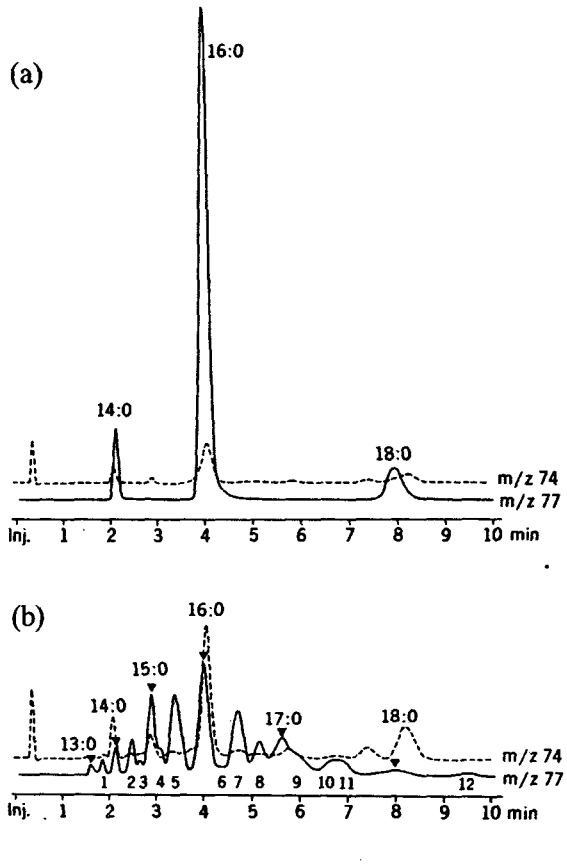


Fig. 2 Electron micrographs of Harderian gland fatty acid synthase.

continuous middle cleft along the major axis. The major and minor axes were approximately 220 Å long and 150 Å, respectively. In a dimer, the subunit has a rod-like structure about 220 Å long and 50 Å wide.



1. 4-methyl-C-13:0
2. 4-methyl-C-14:0
3. 12-methyl-C-14:0
4. 4,13-dimethyl-C-15:0
5. 8-methyl-C-15:0
6. 4,14-dimethyl-C-16:0
7. 4-methyl-C-16:0
8. 14-methyl-C-16:0
9. 6,15-dimethyl-C-17:0 or 8,5-dimethyl-C17:0
10. 8-methyl-C-17:0
11. 4-methyl-C-17:0
12. 4-methyl-C-18:0

Fig. 3 Fatty acids produced by the fatty acid synthase from guinea pig harderian gland. The substrates used in experiment were as follows: (a) acetyl-CoA and malonyl-CoA, (b) acetyl-CoA, malonyl-CoA, and methylmalonyl-CoA.

In the textbook of biochemistry, it is written that the acetyl-CoA is a primer for fatty acid synthesis and malonyl-CoA is an elongating substrate, producing palmitic acid as a product. But our body contains different kind of fatty acids other than palmitic acid. Some are converted by elongation or desaturation from palmitic acid, but fatty acid synthase itself can produce several kinds of fatty acids by the combination of primer and elongating substrate.

We developed a new assay method for fatty acid synthase involving mass fragmentography (3). The enzyme reaction is performed in D_2O. Under the standard incubation conditions with acetyl-CoA and malonyl-CoA as substrates, a large amount of palmitic acid together with small amounts of myristic and stearic acid was synthesized (Fig. 3a). When acetyl-CoA was replaced by propionyl-CoA, both odd-numbered fatty acids (13:0, 15:0, 17:0) and even-numbered fatty acids were produced. When methylmalonyl-CoA was added together with malonyl-CoA, more than 19 kinds of fatty acids were produced (Fig. 3b). Most of the products were methyl-branched fatty acids, but straight-chain acids with even and odd numbers of carbon atoms were also produced (4).

The Harderian gland of golden hamsters excretes alkyldiacylglycerol (ADG), the fatty acid and alkyl compositions of which differ between males and females (5). ADG in males contains mostly straight

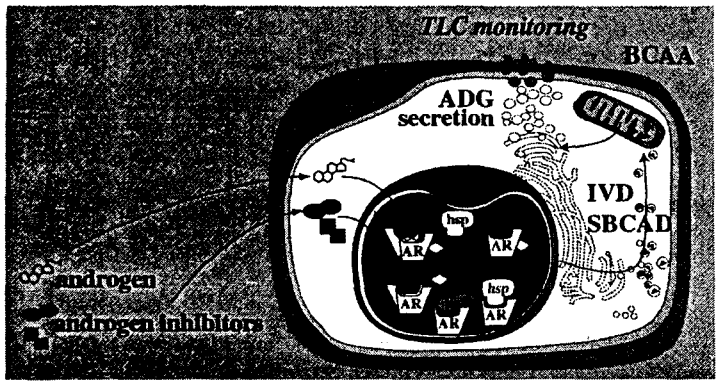


Fig. 4 Hormonal regulation in the Harderian gland.

chain fatty acids, even- and odd-numbered, the major one being 15:0, while ADG in females contains *iso*- and *anteiso*-branched chain acids. Treatment of females with testosterone led the disappearance of such branched chain fatty acids (6). Castration led to the appearance of *iso*- and *anteiso*-branched fatty acids. As *iso*- or *anteiso*-branched chain fatty acids were produced with branched chain acyl-CoAs as primers, we supposed that the androgenic regulation occurred at the step of branched chain amino acid (BCAA) degradation: isovaleryl-CoA dehydrogenase and 2-methylbranched-chain acyl-CoA dehydrogenase.

These fatty acids are synthesized in the cell and not necessary to take from the diet. We can produce most of the fatty acids in the body by ourselves.

Essential Fatty Acids and Leukotrienes

Mammalian cells contain three kinds of desaturase designated delta-9, -6, and -5 fatty acyl-CoA desaturase, but not delta-12 and -15 desaturase. Since mammalian tissues have lost the ability to introduce double bonds beyond the ninth carbon atom of a fatty acid chain, we should ingest linoleic and α-linolenic acid in the diet. These fatty acids are produced in the plant and once we absorb them into the body, we convert them into bioactive fatty acids like prostaglandins. That is why they are called essential fatty acids.

Linoleic acid is converted to arachidonic acid and α-linolenic acid is changed to EPA and DHA by successive reactions of elongase and desaturase.

When arachidonic acid is released from phospholipid by certain stimuli, it is converted to prostaglandins and thromboxanes by the action of cyclooxygenase (cyclic pathway). Alternatively, arachidonic acid is transformed by 5-lipoxygenase (linear pathway) to leukotriene A_4, which is a key intermediate for the syntheses of leukotriene B_4, C_4 and other related peptidyl leukotrienes.

The leukotrienes (LTs) constitute a family of arachidonic acid metabolite involved not only in inflammatory or pathological processes but also in neuroendocrine functions. Leukotriene B_4 is the most potent chemotactic compounds, and it causes chemotaxis, adhesion of leukocytes to the endothelial cells, degranulation, and superoxide anion generation from human neutrophils.

We established the quantitation method of leukotrienes and related compounds by HPLC and GC-MS (7). The latter is more sensitive and specific method, and demonstrated that more than 80% of leukotriene B_4 were rapidly decomposed to its omega-2,3-oxidized products and the major metabolite of leukotriene B_4 is 20-hydroxy-LTB_4.

Then we studied the biosynthesis of leukotrienes (8). We identified the leukotriene A_4 synthase to

arachidonate 5-lipoxygenase. LTA_4 is converted to LTB_4 by LTA_4 hydrolase, and LTC_4 by LTC_4 synthase. We sequenced cDNA coding for human LTA_4 hydrolase, which was the first demonstration of molecular cloning of the enzyme linked to the biosynthesis of eicosanoids (9). The enzyme is composed of 610 amino acids with a molecular weight of 69,153.

We also purified and characterized the LTB_4 receptor from porcine spleen (10).

Platelet Activating Factor, a Bioactive Lipid

Platelet-activating factor (PAF) is an ether-linked phospholipid, having a structure, 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine. It possesses potent proinflammatory, smooth-muscle contractile and hypotensive activities, and appears to be crucial in the pathogenesis of bronchial asthma and in the lethality of endotoxin and anaphylactic shock.

Molecules with biological activities are usually considered to act on cells via interaction with specific receptors, generally on the cell surface. To elucidate the structure of PAF receptor, we used a ligand-specific cloning strategy using gene expression system in *Xenopus laevis* oocyte and electrophysiological detection of PAF-induced responses (11). The protein encoded by the insert of functional phage clone was identified as the PAF receptor based on its pharmacological properties when expressed in oocytes and COS-7 cells.

Cloned DNA had 3,020 nucleotides and deduced amino acid sequence was 342. Hydropathy profile analysis revealed the existence of seven hydrophobic, putative transmembrane segments characteristic of G-protein coupled receptors.

This was the first cloning of a receptor for a lipid mediator, a family including prostaglandins, leukotrienes and lipoxins. The development of such a simple and sensitive assay system of PAF agonist and antagonists and its application to clinical studies will be essential to evaluate the role of PAF in diseases.

PAF is quickly hydrolyzed by PAF acetylhydrolase (PAF-AH) to produce lysoPAF, which is then esterified with arachidonic acid. PAF-AH is considered as the major anti-inflammatory component of lipoprotein.

From Microscopic Observation to Macroscopic Understanding

We started our research on lipid metabolism from the purification of fatty acid synthase, and developed an assay method using mass fragmentography which enabled us to analyze the individual products under different incubation condition. We revealed that the fatty acid synthase can produce almost all fatty acids except essential ones needed in the cell. The production of fatty acids is regulated by several factors, and in some case it is regulated at the step of acyl-CoA dehydrogenase by androgenic control.

Metabolism of lipids in the cell has been analyzed by a biochemical approach in a microscopic scale. Digestion and absorption through the intestinal wall are other phenomenon to be considered, and there is a gap between the lipids in food and those in the cell. But observations in the cell are also applicable to a macroscopic understanding of the role of lipids in nutrition.

Bioactive lipids can exert a definite influence on a target cell as a signal transducer through its specific receptor. Disturbance of this system causes disease like asthma or allergy. The microscopic finding described above may help in the rational design of therapeutic treatment of patients by food for these disease.

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