PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

A Joint Meeting of the Biochemical Society and the Society of Biological Chemists (India) was held at the Institute of Science, Bangalore, on Thursday–Tuesday, 2–7 December 1971, when the following papers were presented:

INTERNATIONAL SYMPOSIUM ON LIPIDS

Enzyme Systems for Fatty Acid Synthesis

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Enzyme systems capable of fatty acid synthesis from malonyl-CoA, called fatty acid synthetases, are widely distributed in Nature and can be isolated from various unicellular organisms, plants and animal tissues. All enzyme systems require, in addition to malonyl-CoA, NADPH as a reducing agent and acetyl-CoA as a primer of the synthetic process.

Fatty acid synthetases may be arranged into two groups: (1) stable multienzyme complexes, having molecular weights between 5×10^5 and 2.3×10^6 , in which all the individual enzymes of the pathway are assembled into a compact unit, e.g. those of yeast and various animal tissues, etiolated *Euglena gracilis*, *Penicillium patulum* and *Mycobacterium phlei*; (2) fatty acid synthetases in which the constituent enzyme readily separate on conventional protein fractionation, e.g. those of *Escherichia coli*, *Clostridium*, *Pseudomonas* and photoauxotrophic *Euglena gracilis*.

Both groups of fatty acid synthetases contain protein-bound 4'-phosphopantetheine (acyl-carrier protein), which serves as a carrier of acyl groups during chain elongation. In yeast, adipose tissue and liver the acyl-carrier protein is integrated into the multienzyme complex. The swinging arm of length about 2.0 nm (20 Å) carries the intermediates bound covalently to its thiol group to and from each of the enzymes, which have little freedom of motion in the rather rigid structure of the multienzyme complex. In contrast, bacterial and plant acyl-carrier proteins are not firmly attached to the individual enzymes involved in fatty acid synthesis. Consequently the fatty acyl derivatives of acyl-carrier protein themselves can migrate and serve as substrates in subsequent reactions. In the stable multienzyme complexes the fatty acids must be released from the acyl-carrier protein component associated with the complex either by transfer to CoA (in yeast) or by hydrolysis (in animal tissues).

All synthetases produce fatty acids of different chain length. With the yeast enzyme system the distribution of fatty acids can be described in terms

of a mathematical model, based on the assumption that the fatty-acyltransferase involved undergoes hydrophobic interaction with the growing carbon chain, starting at the level of the C_{13} acid.

Fatty acid synthetases of the first group may be visualized as compartments in which all the intermediates are covalently bound and in which contact with the outside is mediated by the various acyltransferases. Consequently the specificity and multiplicity of the acyltransferases play a decisive role in this kind of compartmentation. In the yeast system acetyl-CoA, in its function as a 'primer', can be replaced by homologous saturated acyl-CoA derivatives. It was found that nonanoyl-CoA and decanoyl-CoA were good 'primers' at low concentrations. At higher concentrations of the 'priming' substrate an inhibitory effect became apparent with all long-chain acyl-CoA derivatives. Studies with decanoyl-CoA showed that this inhibition could be interpreted as being due to competition between decanoyl-CoA and malonyl-CoA for the same acceptor site. The rate of synthesis with DL-3-hydroxydecanoyl-CoA and dec-2-enoyl-CoA as 'primer' was 25–50-fold lower than that with decanoyl-CoA, owing to the slow transfer of the acyl residue from CoA to the acyl-carrier protein. However, the 'priming' activity of derivatives of decanoyl-CoA with modifications at positions more distant from the carboxyl group, such as dec-3-enoyl-CoA or dec-9-enoyl-CoA or 4-oxodecanoyl-CoA, was depressed to a much smaller extent. In these cases the functional groups were retained during chain elongation.

The active site of the various acyltransferases contains serine residues that act as carriers between CoA and the acyl-carrier protein. A stable [14 C]-malonyl-pentapeptide containing serine, histidine, glycine, alanine and leucine was isolated from the peptic hydrolysate of [14 C]malonyl-enzyme. Tryptic digestion of the [14 C]acetyl-enzyme led to the isolation and characterization of the two stable [14 C]acetyl-peptides:

[14 C]Ac-Ser-Gln-Gly-Leu-Val-Thr-Ala-Val
and
[14 C]Ac-Ser-Gln-Gly-Leu-Val-Thr-Ala

The absence of basic amino acids from these peptides indicates that they are derived from the C-terminal ends of polypeptide chains. It is assumed that the

native acetyltransferase component of the enzyme complex contains at its C-terminus a valine residue that is partly removed by proteolysis during the isolation of fatty acid synthetase from yeast cells.

With respect to the genetics of the fatty acid synthetase complex from yeast, experiments by Schweizer *et al.* (1971) will be reviewed that suggest that several faggenes are linked and co-ordinatedly transcribed.

Schweizer, E., Kuhn, L. & Castorph, H. (1971) *Hoppe-Seyler's Z. Physiol. Chem.* **352**, 377

Regulatory Mechanisms for Liver Acetyl-Coenzyme A Carboxylase

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Acetyl-CoA carboxylase plays a critical role in the control of fatty acid synthesis. Several lines of evidence indicate that the regulation at this carboxylation step is achieved by changes both in the quantity of the carboxylase and in the catalytic efficiency per enzyme molecule (see Numa *et al.*, 1970). It appears that the former mechanisms are more involved in the long-term control, whereas the latter mechanisms are more responsible for the short-term control.

Previous studies (Numa *et al.*, 1969; Majerus & Kilburn, 1969; Nakanishi & Numa, 1970) disclosed that the variations in the level of acetyl-CoA carboxylase activity in liver extracts derived from rats under different dietary and hormonal conditions are determined by changes in the quantity of the carboxylase protein. It was also shown that the increase or decrease in the carboxylase quantity in re-fed or alloxan-diabetic rats is due to differences in the rate of enzyme synthesis, whereas the decrease in the carboxylase quantity in starved animals is due both to diminished enzyme synthesis and to accelerated enzyme degradation. In the present work we have attempted to define the mechanisms underlying the increase in the level of liver acetyl-CoA carboxylase activity in genetically obese mice (C57BL/6J-*ob*). It was demonstrated that there were no qualitative differences between the enzymes derived from obese mice and non-obese mice (C57BL/6J), in terms of immunological and kinetic properties. Combined immunochemical and isotopic techniques revealed that the rate of enzyme synthesis per liver was 7.7-fold higher in obese mice than in non-obese mice. The rate constant of enzyme degradation was found to be 1.7-fold smaller in obese mice than in non-obese mice, the half-lives being 115h and 67h

respectively. These results indicate that the increase in the content of liver acetyl-CoA carboxylase in obese mice is due mainly to a rise in the rate of enzyme synthesis and in a minor degree to a decrease in the rate constant of enzyme degradation.

To investigate the mechanisms by which the catalytic efficiency of acetyl-CoA carboxylase molecules is controlled, we made kinetic studies on the reaction mechanism as well as on the allosteric activation by citrate. The rat liver enzyme purified to homogeneity were employed. Previous results of initial-velocity and product-inhibition studies (Hashimoto *et al.*, 1970; Hashimoto & Numa, 1971) indicated that the carboxylase reaction proceeds through the Bi Bi Uni Uni Ping Pong mechanism, the order of addition of substrates being ATP, HCO_3^- and acetyl-CoA in the forward reaction, and malonyl-CoA, P_i and ADP in the reverse reaction. It was also shown that the site of citrate action during the carboxylase catalysis lies predominantly on the carboxylated enzyme, the dissociation constant of the carboxybiotinyl-enzyme-citrate complex being 3–5 mM. In the present investigations the reaction mechanism was corroborated by analysis of the inhibition patterns obtained with kynurenate, which was found to be a novel dead-end inhibitor acting on the uncarboxylated enzyme. The site of citrate action during the carboxylase catalysis was confirmed by studies on initial rates of malonyl-CoA- ^{14}C acetyl-CoA exchange. On the other hand the dissociation constant of the uncarboxylated enzyme-citrate complex was estimated to be 3–14 μM in terms of ^{14}C citrate binding, protection against avidin inactivation, protection against trypsin activation and shift in the protomer-polymer equilibrium. This explains why the citrate effect on the uncarboxylated enzyme does not manifest itself during the carboxylase catalysis. These findings also indicate that the carboxylation of the biotinyl prosthetic group gives rise to a conformational change at the citrate site, resulting in a marked decrease in the affinity of the enzyme for the activator.

Hashimoto, T. & Numa, S. (1971) *Eur. J. Biochem.* **18**, 319

Hashimoto, T., Iritani, N., Nakanishi, S. & Numa, S. (1970) *Proc. Japanese Conf. Biochem. Lipids 12th. Meet., Ikahio*, p. 21

Majerus, P. W. & Kilburn, E. (1969) *J. Biol. Chem.* **244**, 6254

Nakanishi, S. & Numa, S. (1970) *Eur. J. Biochem.* **16**, 161

Numa, S., Nakanishi, S. & Iritani, N. (1969) *Proc. Japanese Conf. Biochem. Lipids 11th. Meet., Sapporo*, p. 235

Numa, S., Nakanishi, S., Hashimoto, T., Iritani, N. & Okazaki, T. (1970) *Vitam. Horm. (New York)* **28**, 213