Molecular Aspects of Fatty Acid Synthesis

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Part 1: Acetyl-CoA Carboxylase

The role of phosphorylation/dephosphorylation of acetyl-CoA carboxylase in the regulation of mammalian fatty acid biosynthesis

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The main lipogenic tissues of the rat are the liver, white adipose tissue and, during lactation, the mammary gland. The rate of fatty acid synthesis de novo in these tissues must be regulated according to the nutritional and physiological status of the animal. Such short-term regulation is primarily achieved through the action of hormones. Acetyl-CoA carboxylase is a prime target for such hormonal regulation as it catalyses the rate-limiting step in the conversion of cytoplasmic acetyl-CoA to fatty acid. While acetyl-CoA carboxylase can be regulated allosterically by citrate (activator) and fatty acyl-CoA (inhibitor), it is becoming clear that phosphorylation and dephosphorylation, leading to inactivation and activation respectively, play a major part in the hormonal regulation of this enzyme activity.

To examine the effects of hormones on phosphorylation of acetyl-CoA carboxylase, we have labelled isolated hepatocytes or adipocytes to steady state with [32P]phosphate, treated the cells with or without hormone, and have then purified the enzyme to homogeneity using affinity chromatography on immobilized avidin in the presence of protein kinase and phosphatase inhibitors (NaF and EDTA). We simultaneously make per-chloric acid extracts of the cells and analyse the specific radioactivity of cellular adenine nucleotides in these extracts using a h.p.l.c. method (Sharps et al., 1979). In every case the stoichiometry of phosphorylation of acetyl-CoA carboxylase in the intact cell may be estimated. The validity of these estimations has been confirmed in the case of hepatocytes by making spectrophotometric determinations (Ames, 1966) of alkali-labile phosphate for enzyme purified from hepatocytes or intact liver (Holland et al., 1984).

In vivo, glucagon inhibits hepatic fatty acid synthesis by approx. 60% (Cook et al., 1977), and a similar inhibition is seen after glucagon treatment of isolated hepatocytes (Fig. 1). This correlates with an equivalent decrease in acetyl-CoA carboxylase activity measured in crude cell extracts at 0.5 mMCitrate, which we estimate to be the approximate physiological concentration of this allosteric activator. The effect of glucagon survival purification of the enzyme, and is accompanied by a significant increase in total enzyme phosphate content (0.8 mol/subunit), which occurs over and above a remarkably high basal level of phosphorylation (Fig. 1).

In adipocytes, adrenaline causes a 40–50% decrease in acetyl-CoA carboxylase activity measured in crude extracts (Brownsey et al., 1979; Haystead & Hardie, 1986), although this is not reflected in overall rates of lipogenesis from glucose, probably because the hormone has the opposite effect on conversion of glucose to citrate (Cahill et al., 1960; Halestrap & Denton, 1974). Glucagon causes a 70% decrease in acetyl-CoA carboxylase activity in crude cell extracts (Zammit & Corstrorphine, 1982) and the effects of both adrenaline and glucagon on enzyme activity measured at 0.5 mMCitrate are still evident after purification on avidin-Sepharose (Fig. 1). These hormones also cause very significant increases in the total phosphate content of the enzyme (Fig. 1).

The hormone-induced increases in phosphorylation of acetyl-CoA carboxylase in isolated adipocytes and hepatocytes are even more evident when chromatographic digests of [32P]-labelled enzyme are analysed by reversed-phase h.p.l.c. (Holland et al., 1984, 1985). In every case the increase in phosphorylation is largely accounted for by a single chromotryptic peptide which is identical with the peptide containing the major site phosphorylated on the purified enzyme by cyclic AMP-dependent protein kinase. We have previously shown that phosphorylation at this site by cyclic AMP-dependent protein kinase inactivates the enzyme, an effect which is reversed by dephosphorylation (Hardie & Guy, 1980; Munday & Hardie, 1984).

Since adrenaline (in adipocytes) and glucagon (in adipocytes and hepatocytes) are known to elevate cytosolic cyclic AMP concentrations and activate cyclic AMP-dependent protein kinase, our results provide strong evidence that these hormones inhibit acetyl-CoA carboxylase via a direct phosphorylation mechanism.
The most important positive signal for lipogenesis is believed to be insulin. However, recent studies have suggested that insulin and growth factors such as epidermal growth factor (EGF) or insulin-like growth factor-1 may act via common mechanisms (e.g. Ullrich et al., 1985). It is therefore of interest that insulin and EGF both stimulate fatty acid synthesis in hepatocytes (≈40%; Holland & Hardie, 1985) and adipocytes (15-fold and 4-fold respectively; Haystead & Hardie, 1986). It is clear that activation of acetyl-CoA carboxylase plays a part in this stimulation, since treatment of adipocytes with insulin or EGF causes increases in acetyl-CoA carboxylase activity measurable in cell extracts (Halestrap & Denton, 1973; Haystead & Hardie, 1986). Contrary to expectations, this increased activity is not associated with a dephosphorylation of the enzyme, but occurs in conjunction with an increased phosphorylation at a site distinct from that phosphorylated by cyclic AMP-dependent protein kinase (Brownsey & Denton, 1982; Witters et al., 1983; Holland & Hardie, 1985). However, although increased phosphorylation of the enzyme is still observed after purification on avidin-Sepharose from either adipocytes or hepatocytes, the effect of insulin on enzyme activity disappears during purification (Witters et al., 1983; Holland & Hardie, 1985). Two further recent observations in our laboratory cast doubt on the hypothesis that the insulin-stimulated phosphorylation of acetyl-CoA carboxylase causes enzyme activation:

1. The effect of insulin treatment of adipocytes on enzyme activity is not reversed by addition of a large amount of protein phosphatase-2A to crude cell extracts. We have confirmed, using extracts from 32P-labelled cells, followed by enzyme purification, that the phosphatase treatment completely dephosphorylated all labelled sites on acetyl-CoA carboxylase [including the insulin-sensitive 'I' site described by Brownsey & Denton (1982)].

2. The effect of insulin on enzyme activity is lost if crude cell extracts are rapidly gel-filtered by centrifugation through Sephadex G-25 in the presence of 0.5 M-NaCl. Addition of 0.5 M-NaCl without gel filtration, or gel filtration at low ionic strength, has no effect. These results suggest that activation of acetyl-CoA carboxylase by insulin in adipocytes may be due to changes in the concentration of low molecular weight effector(s) which bind tightly to the enzyme at low ionic strength.

In a different tissue, the lactating rat mammary gland, it appears that insulin may be acting through an entirely different mechanism, i.e. a dephosphorylation of acetyl-CoA carboxylase. At peak lactation, the rat mammary gland is extremely active in the synthesis of fatty acids de novo. This makes considerable demands upon maternal substrate supplies and requires stringent regulation according to the nutritional state of the mother. Thus 24 h starvation produces a 98% decrease in the rate of mammary gland lipogenesis. This inhibition can be rapidly and totally reversed by refedding the rat with chow diet for 2.5h (Robinson et al., 1978; Fig. 2). These changes correlate well with changes in the circulating insulin concentration, and streptozotocin administration to 24 h starved animals immediately before refedding blocks the reactivation, indicating that insulin is the signal (Robinson et al., 1978; Fig. 2). Acetyl-CoA carboxylase catalyses one of a number of enzymic steps likely to be important in the regulation of mammary gland fatty acid synthesis from glucose, including also glucose transport, hexokinase, phosphofructokinase and pyruvate dehydrogenase. The activity of acetyl-CoA carboxylase in crude mammary gland extracts changes in parallel with the changes in lipogenic rate shown in Fig. 2 (Munday & Williamson, 1982) and we have demonstrated that this is due to covalent modification of the enzyme. When measured at physiological citrate concentrations, the activity of acetyl-CoA carboxylase puri-
Fig. 2. Rates of lipogenesis and the activity and phosphate content of acetyl-CoA carboxylase purified from the lactating rat mammary gland

All experiments were performed at 10:00 h, and the treatment of the rats was as follows: (F) controls fed chow ad libitum, (S) 24 h starved, (RF) starved-2.5 h refed, and (RFSt) 24 h starved-2.5 h refed-2.5 h streptozotocin-treated (Schein et al., 1971). Fatty acid synthesis was measured in vivo as described by Munday & Williamson (1981). Acetyl-CoA carboxylase (ACC) was purified from the lactating rat mammary gland by avidin-Sepharose chromatography and its activity was measured by the HICO₂ fixation method (Munday & Hardie, 1984). The alkali-labile phosphate content of the enzyme was measured spectrophotometrically (Ames, 1966). The heights of the bars represent means from the number of observations in parentheses and the vertical lines represent standard errors.

Refeeding chow diet for 2.5 h restores the activity of the enzyme to control levels, and is accompanied by a loss of approximately 0.8 mol of phosphate per mol of subunit (Fig. 2). This effect of refeeding can be mimicked in vitro by treating acetyl-CoA carboxylase purified from the mammary glands of 24 h starved lactating rats with the catalytic subunit of protein phosphatase 2A (purified from rabbit skeletal muscle). This treatment results in a time-dependent dephosphorylation and activation of the acetyl-CoA carboxylase such that its activity is indistinguishable from that of enzyme from fed controls also treated with phosphatase. Just as the reactivation of mammary gland lipogenesis by refeeding is blocked by streptozotocin treatment, so too is the reactivation of acetyl-CoA carboxylase (Fig. 2). Thus we have evidence that in the lactating mammary gland insulin can promote the dephosphorylation and activation of acetyl-CoA carboxylase, representing one component of the stimulation of fatty acid synthesis by the hormone. Starvation for 6 h or short-term (2 h) streptozotocin treatment of fed animals are both known to rapidly decrease plasma insulin concentrations and cause marked inhibition (80%) of mammary gland lipogenesis (Robinson et al., 1978; Williamson et al., 1983; Jones et al., 1984). However, neither of these treatments had an effect on the activity of acetyl-CoA carboxylase purified from the lactating gland. It is presumed that one or more of the other enzymic steps (mentioned previously) are important in this short-term inhibition of mammary lipogenesis. The phosphorylation and inactivation of mammary gland acetyl-CoA carboxylase in vivo appears to be somewhat slower, but may account for the further dramatic inhibition of lipogenesis (from 80% to 98% inhibition of fed control values) that occurs between 6 h and 24 h starvation.

The lactating rat mammary gland is not sensitive to the hormones that raise cyclic AMP concentrations in liver and adipose tissue. Thus lipogenesis is unaffected by adrenaline or glucagon either in the mammary gland in vivo (Bussman et al., 1984; Jones et al., 1984) or in isolated acini in vitro (Williamson et al., 1983; Robson et al., 1984). This is despite the presence of the necessary enzymic activities, i.e. adenylate cyclase (Bar, 1973), phosphodiesterase (Mullaney & Clegg, 1984) and cyclic AMP-dependent protein kinase (Burchell et al., 1978). There is evidence that mammary acinar cells lack receptors for glucagon (Robson et al., 1984). However, β₁ receptors have been identified, but only in the presence of phosphodiesterase inhibitors can β₁ agonists increase cyclic AMP concentrations to any significant extent (Clegg & Mullaney, 1985). The phosphorylation and inhibition of mammary acetyl-CoA carboxylase in response to prolonged starvation is therefore presumably mediated by a cyclic AMP-independent protein kinase.
Table 1. Protein kinases that phosphorylate purified mammary gland acetyl-CoA carboxylase in vitro

<table>
<thead>
<tr>
<th>Protein kinase</th>
<th>Alkaline phosphate content (mol of phosphate incorporated per mol of enzyme subunit)</th>
<th>Effect on acetyl-CoA carboxylase activity measured at 0.5 mM-maleate</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Cyclic AMP-dependent protein kinase (rabbit muscle)</td>
<td>1.3</td>
<td>75%</td>
</tr>
<tr>
<td>(2) Acetyl-CoA carboxylase kinase-2</td>
<td>0.7</td>
<td>75%</td>
</tr>
<tr>
<td>(3) Casein kinase-1</td>
<td>0.7</td>
<td>None</td>
</tr>
<tr>
<td>(4) Casein kinase-2</td>
<td>0.4</td>
<td>None</td>
</tr>
<tr>
<td>(5) Ca^{2+}-calmodulin-dependent multifunctional protein kinase (rabbit muscle)</td>
<td>1.4</td>
<td>None</td>
</tr>
<tr>
<td>(6) Ca^{2+}-phospholipid-dependent protein kinase (rat brain)</td>
<td>0.9</td>
<td>35%</td>
</tr>
</tbody>
</table>

The protein kinases that have been shown to phosphorylate mammary gland acetyl-CoA carboxylase in vitro are shown in Table 1. Of these protein kinases, nos. 1-4 have been partially purified and characterized from the lactating mammary gland (Munday & Hardie, 1984) while a Ca^{2+}-calmodulin-dependent protein kinase has been identified in rat mammary gland acinar cell preparations (Brooks & Landt, 1985). Casein kinase-1 and -2 phosphorylate sites located on distinct tryptic peptides of acetyl-CoA carboxylase, without affecting the activity of the enzyme (Munday & Hardie, 1984). This is of particular interest since the peptide phosphorylated by casein kinase-2 co-migrates with the peptide containing the insulin-stimulated site on hepatocyte acetyl-CoA carboxylase, both on reversed-phase h.p.l.c. and thin-layer isoelectric focusing (Holland & Hardie, 1985). Ca^{2+}-calmodulin-dependent protein kinase phosphorylates site(s) located in the same tryptic peptide and also has no effect on acetyl-CoA carboxylase activity. It is not yet clear whether the sites phosphorylated by these two protein kinases and in response to insulin within this peptide are identical. Acetyl-CoA carboxylase kinase-2 and cyclic AMP-dependent protein kinase are distinct enzymes with respect to their apparent molecular weights, substrate specificities and the insensitivity of acetyl-CoA carboxylase kinase-2 to the specific protein inhibitor of cyclic AMP-dependent protein kinase (Munday & Hardie, 1984). However, both kinases phosphorylate acetyl-CoA carboxylase in a reversible manner and both produce similar inhibitions of enzyme activity (Munday & Hardie, 1984; Table 1). Phosphorylation of acetyl-CoA carboxylase by these two kinases produced very similar patterns of either chymotryptic or tryptic phosphopeptides. The major chymotryptic or tryptic phosphopeptide of each kinase co-migrates both on reversed-phase h.p.l.c. and thin-layer isoelectric focusing. The Ca^{2+}- and phospholipid-dependent protein kinase phosphorlates three distinct tryptic peptides of acetyl-CoA carboxylase. One of these is the major tryptic peptide phosphorylated by cyclic AMP-dependent protein kinase and acetyl-CoA carboxylase kinase-2. This may account for the inactivation of acetyl-CoA carboxylase observed after phosphorylation by this kinase (Table 1), an effect which is reversed by protein phosphatase treatment.

Our present investigations include analysis of the peptides for the amino acid residues within the peptides that are phosphorylated in vivo and by the protein kinases in vitro; this analysis may enable us to establish which, if any, of these protein kinases are responsible for the phosphorylation and inactivation of mammary gland acetyl-CoA carboxylase in response to physiological situations such as starvation.

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References