Bimodal Activation of Acetyl-CoA Carboxylase by Glutamate*

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Acetyl-CoA carboxylase (ACC) catalyzes the formation of malonyl-CoA, an essential substrate for fatty acid biosynthesis and a potent inhibitor of fatty acid oxidation. Here, we provide evidence that glutamate may be a physiologically relevant activator of ACC. Glutamate induced the activation of both major isoforms of ACC, prepared from rat liver, heart, or white adipose tissue. In agreement with previous studies, a type 2A protein phosphatase contributed to the effects of glutamate on ACC. However, the protein phosphatase inhibitor micocystin LR did not abolish the effects of glutamate on ACC activity. Moreover, glutamate directly activated purified preparations of ACC when protein phosphatase activity was excluded. Phosphatase-independent ACC activation by glutamate was also reflected by polymerization of the enzyme as judged by size-exclusion chromatography. The sensitivity of ACC to direct activation by glutamate was diminished by treatment in vitro with AMP-activated protein kinase or cAMP-dependent protein kinase or by β-adrenergic stimulation of intact adipose tissue. We conclude that glutamate, an abundant intracellular amino acid, induces ACC activation through complementary actions as a phosphatase activator and as a direct allosteric ligand for dephosphorylated ACC. This study supports the general hypothesis that amino acids fulfill important roles as signal molecules as well as intermediates in carbon and nitrogen metabolism.

Malonyl-CoA might also contribute to the regulation of pancreatic β-cell secretion (11, 12).

Two major mammalian ACC isoforms and additional splice variants have been recognized. ACC-α (ACC-1, subunit Mr 265,000) and ACC-β (ACC-2, subunit Mr 280,000) are products of distinct genes that display discrete tissue expression patterns and physical and enzymatic properties (13–19). ACC activity and cellular malonyl-CoA concentrations change rapidly in response to hormone treatment of cells. ACC is activated following treatment in vitro of fat or liver cells with insulin or by hyperinsulinemia in vivo and is rapidly deactivated when cells or tissues are exposed to catecholamines or glucagon (2–4, 20, 21). Similarly, malonyl-CoA levels rise in insulin-treated muscle and heart and are decreased by counter-regulatory hormones, exercise, or ischemic stress (6–10, 22, 23).

Control of ACC activity reflects the actions of allosteric modulators and of protein kinases and phosphatases that control the phosphorylation state of key serine residues (2–4). These regulatory mechanisms probably influence ACC activity by altering the equilibrium between inactive ACC dimers and highly active polymers (2–4, 24–26). Physiologically relevant allosteric activators of ACC include tricarboxylic acids such as citrate, whereas coenzyme A and CoA esters are potent inhibitors (24, 25, 27, 28). AMPK appears to play a dominant role in mediating inhibition of ACC-α, notably through phosphorylation of serine 79, with possible contribution from phosphorylation of serine 1200 (3, 29). Corresponding regulatory sites on ACC-β have not yet been defined, although this isoform is an excellent substrate for PKA as well as for AMPK (15, 30–32) and is phosphorylated on multiple sites within intact cardiac myocytes (32).

The roles of malonyl-CoA and ACC in fatty acid synthesis and β-oxidation illustrate important features of the regulatory interplay between lipid and carbohydrate metabolism in mammals that extend the concepts embodied in the glucose-fatty acid cycle (33). Amino acids, as well as lipids and carbohydrates, are crucial for intermediary metabolism, providing an important source of carbon for energy metabolism and for the synthesis of glucose and glycerogen (e.g. see Refs. 34 and 35). Furthermore, amino acid carbon can be utilized for fatty acid synthesis, either directly through the generation of keto acids or acetyl-CoA or indirectly following gluconeogenesis (36–38). Despite the important metabolic contributions of amino acids, we know rather less about the possible regulatory roles of amino acids, especially in the context of fatty acid metabolism. Recently, a glutamate-sensitive protein phosphatase was recognized in rat liver that was able to dephosphorylate and activate ACC (39, 40). This protein phosphatase appears to account for the activation of ACC and lipogenesis observed following the treatment of isolated hepatocytes with glutamine (39, 40). In view of the quantitative importance of glutamine in amino acid metabolism and the abundance of intracellular glutamate in many cells (35), we examined the possibility that ACC control might be sensitive to glutamate in tissues other

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Previous reports have demonstrated activation of ACC by treatment of intact rat hepatocytes with glutamine or by addition of sodium glutamate to cellular supernatant fractions (39, 40). We confirmed the glutamate sensitivity of rat liver ACC and found that it persisted following partial purification of the enzyme from rat liver by ammonium sulfate precipitation and centrifuged just prior to chromatography (315,000 × g, 15 min, 4 °C). The supernatant was incubated in the presence of microcystin LR (2 μM) and glutamate or citrate (as described above) for 30 min at 37 °C. Samples (250 μl) were subjected to chromatography at room temperature (10 ml/h); 1-ml fractions were collected; and ACC activity was determined.

RESULTS

Glutamate Activates ACC Isoforms from Rat Liver, Heart, and White Adipose Tissue

experiments.

Activation of ACC by Glutamate Is Only Partially Inhibited by Microcystin LR—Previous studies have shown that the effects of glutamate on hepatic ACC are essentially abolished by

than liver. We demonstrate that glutamate does stimulate a phosphatase-mediated activation of ACC in subcellular fractions from heart and adipose tissue as well as liver. Unexpectedly, the effects of glutamate on ACC activity were not completely inhibited by the protein phosphatase inhibitor microcystin LR. This observation led to further studies that confirmed direct, allosteric effects of glutamate on both major ACC isoforms. These observations suggest that amino acids have important and complex effects on intermediary metabolism, involving actions as signal molecules as well as sources of carbon and nitrogen.

EXPERIMENTAL PROCEDURES

Materials—Laboratory chemicals, biochemicals, and solvents were obtained as described previously (32). t-Glutamate was obtained from Sigma. Microcystin LR was from Calbiochem. Custom peptides including the “SAMS” peptide for assay of AMPK (HRRSAMSGLHLVKRR) was synthesized by the Nucleic Acid and Protein Service Laboratory (University of British Columbia).

Purification of ACC—Male Wistar rats, maintained on a 12-h light/dark cycle and allowed free access to laboratory chow, were killed by cervical dislocation or by asphyxiation in carbon dioxide, and tissues were weighed immediately. Livers and hearts were obtained from rats weighing 200–400 g and were chilled on ice prior to homogenization. Perirenal and epididymal fat pads from rats weighing 180–240 g were either placed on ice or rinsed and then incubated with shaking (30 min, 37 °C) in oxygenated and bicarbonate-buffered medium (41).

Assay of ACC Activity—Liver and adipose tissue ACC activities were assayed by [14C]bicarbonate fixation (43, 44). Prior to assay, ACC was preincubated (20 min, 37 °C) in pH 7.2 buffer containing MOPS (20 mM), sucrose (250 mM), EDTA (2 mM), EGTA (2 mM), benzamidine (2.5 mM), pepstatin A (3 μM), leupeptin (5 μM), glutathione (2.5 mM), and phenylmethylsulfonyl fluoride (0.5 mM). All procedures were carried out on ice or at 4 °C unless indicated otherwise. A glass-Teflon Potter-Elvehjem homogenizer was used to homogenize livers, and a Polytron was used for hearts and adipose tissue. The homogenates were centrifuged at 10,000 × g for 5 min and then at either 250,000 × g for 60 min (liver) or 315,000 × g for 15 min (heart and adipose tissue). ACC was recovered from the high-speed supernatant fraction by precipitation with ammonium sulfate (40% saturation), resuspended in the initial homogenization buffer, and subjected to chromatography on a monomeric avidin affinity column (15, 42). Biotin-eluted proteins were concentrated to ∼0.5 mg/ml by centrifugation (50-kDa filters, Millipore Corp.) and stored in aliquots at −70 °C.

Assay of ACC Activity—Liver and adipose tissue ACC activities were assayed by [14C]bicarbonate fixation (43, 44). Prior to assay, ACC was preincubated (20 min, 37 °C) in pH 7.2 buffer containing MOPS (20 mM), bovine serum albumin (2 mg/ml), and EDTA (0.5 mM). Other additions to preincubations, where indicated, included microcystin LR (1 μM) or MeOH vehicle (1%, v/v) and the allosteric regulators trisodium citrate and/or sodium glutamate and magnesium acetate. Samples of preincubated ACC were then transferred to assay buffer and incubated for 5–10 min at 37 °C. One unit of ACC catalyzes the formation of 1 μmol of malonyl-CoA/min.

Because of the low abundance in rat hearts, the activity of myocardial ACC was assayed by determination of malonyl-CoA following reverse-phase HPLC. ACC was preincubated as described above and then assayed in a final volume of 100 μl for 10 min at 37 °C. The reaction was stopped with 7 μl of perchloric acid (60%, v/v); the precipitated proteins were removed by centrifugation (10,000 × g, 15 min); and aliquots (20 μl) were subjected to HPLC analysis as described (32).

Phosphorylation of Protein Kinases—AMPK (from rat liver) and PKA (from beef heart) were purified and assayed as described previously (32, 45, 46). Protein determinations were carried out with the dye binding assay using IgG as a standard (47).

Phosphorylation and Dephosphorylation of ACC—Phosphorylation of affinity-purified ACC was achieved by incubation with either PKA or AMPK plus AMP (200 μM) for 1 h at 37 °C. Phosphorylation reactions were carried out in pH 7.2 buffer containing MOPS (20 mM), EDTA (0.5 mM), magnesium acetate (2 mM), and ATP (100 μM) and were quenched with EDTA (5 mM) prior to ACC assay.

To induce ACC phosphorylation in situ, adipose tissue was incubated with Krebs-Henseleit buffer as described above (2 × 15 min, 37 °C) and then for a further 20 min in fresh buffer supplemented with defatted bovine serum albumin (1%, v/v) in the presence of isoproterenol (500 nM). The tissue was blotted, frozen in liquid nitrogen, and homogenized in buffer containing chelators and microcystin LR (1 μM) to inhibit subsequent dephosphorylation. The homogenates were centrifuged (15,000 × g, 5 min, 4 °C), and ACC activity was determined as described above.

To induce ACC dephosphorylation, the enzyme was purified by ammonium sulfate precipitation, resuspended in the initial homogenization buffer plus NaCl (150 mM), and then incubated at 37 °C for 90 min to facilitate the actions of endogenous phosphatases. Proteolytic modification of ACC was prevented by inclusion of leupeptin (5 μM), pepstatin (3 μM), benzamidine (2.5 mM), and Pefabloc SC (0.4 mM). Further affinity purification was as described above.

Analysis of ACC Polymerization by Size-exclusion Chromatography—A Sepharose CL-4B column (0.7 × 48 cm) was equilibrated at room temperature with pH 7.2 buffer containing MOPS (20 mM), EDTA (2 mM), glycerol (2%, v/v), and, when appropriate, either sodium glutamate (50 mM) or sodium citrate (20 mM). Theionic strength of the buffers was kept constant by addition of appropriate concentrations of sodium chloride. ACC was purified from rat liver by ammonium sulfate precipitation and centrifuged just prior to chromatography (315,000 × g, 15 min, 4 °C). The supernatant was incubated in the presence of microcystin LR (2 μM) and glutamate or citrate (as described above) for 30 min at 37 °C. Samples (250 μl) were subjected to chromatography at room temperature (10 ml/h); 1-ml fractions were collected; and ACC activity was determined.

Activation of ACC by glutamate was again apparent at all concentrations of glutamate examined. The effects of glutamate on hepatic ACC are essentially abolished by the Kc for citrate was not significantly altered. In agreement with the previous reports, the effects of glutamate were time-dependent (reaching a maximum within 15 min) and dose-dependent over the range 0–100 mM glutamate (data not shown).

In view of the important metabolic roles and distinct isoform patterns of ACC in non-hepatic tissues, we next tested the ability of glutamate to influence ACC extracted from heart muscle (predominantly ACC-β) or from white adipose tissue (exclusively ACC-α). ACC isolated from heart and white adipose tissue by ammonium sulfate precipitation was also activated upon incubation with glutamate (Fig. 1, B–D, circles; and Table I, part A). In these tissue preparations, the activation of ACC by glutamate was again apparent at all concentrations of citrate examined. The effects of glutamate on the activity of ACC from white adipose tissue were apparent if the tissue was obtained directly from the animal (Fig. 1C) or if it was incubated prior to homogenization (Fig. 1D). ACC obtained from preincubated tissue was more sensitive to citrate than that obtained from non-incubated tissue (Fig. 1, C and D; and Table I), but was still further activated in the presence of glutamate.

It is important to note that the effects of glutamate, observed in these and subsequent experiments, could not be explained by traces of citrate. Direct analysis of stock glutamate solutions (200 mM), using citrate lyase coupled with malate dehydrogenase, revealed very low levels of citrate (<5 μM), far below the Kc for ACC activation (>2 mM) (Table I).

Evidence for Phosphatase-independent Effects of Glutamate on ACC Activity

Activation of ACC by Glutamate Inhibited by Microcystin LR—Previous studies have shown that the effects of glutamate on hepatic ACC are essentially abolished by
microcystin LR, a potent inhibitor of protein phosphatases 2A and 1 (39, 40). As anticipated, microcystin LR significantly attenuated the activation of ACC induced by glutamate treatment (Fig. 1, squares; and Table I). Although the effects of glutamate on ACC were diminished by addition of the protein phosphatase inhibitor, they were not completely eliminated. The effects of glutamate on ACC activity were therefore still significant even in the presence of microcystin LR, with 3-fold activation of ACC still apparent at low citrate concentrations (Fig. 1 and Table I).

Glutamate Activates Purified ACC in the Absence of Protein Phosphatase Activity—In view of the incomplete inhibition of the effects of glutamate by microcystin LR, we explored the effects of glutamate on ACC purified by affinity chromatography to remove endogenous protein phosphatases. As with the enzyme partially purified by ammonium sulfate precipitation, affinity-purified ACC from rat liver was indeed sensitive to glutamate, being maximally activated 3–6-fold by 75 mM glutamate and half-maximally activated by 25 mM glutamate (Fig. 2, squares).

The selectivity of the actions of glutamate on ACC was examined in two respects. In one set of experiments, a variety of organic acids were tested, each at several concentrations, for the ability to activate ACC. The acids tested (with maximum concentrations in parentheses) were aspartate (30 mM), glutamine (5 mM), oxalacetate (65 mM), oxoglutarate (1.6 mM), malate (5 mM), lactate (11 mM), β-hydroxybutyrate (7 mM), acetoacetate (0.8 mM), glucose 6-phosphate (10 mM), and glucosamine 6-phosphate (10 mM). None of these organic acids had significant effects on ACC activity even at the highest indicated values, which exceeded typical intracellular concentrations by at least 4-fold. We were also concerned that the effects of glutamate might be explained by changes in ionic strength. To reproduce the effects observed in previous studies (39, 40), the initial experiments examined the effects of sodium glutamate with no compensation for ionic strength (Fig. 1 and Table I). Other salts (including sodium or potassium chloride), sodium phosphate, and the buffers MOPS and HEPES all failed to activate ACC when tested at concentrations from 25 to 100 mM. Salts containing chloride actually caused ACC inhibition (~30% inhibition at 100 mM NaCl), as reported previously (48). Nevertheless, subsequent experiments were carried out using a buffer from which salt could be removed to compensate for addition of sodium glutamate. Glutamate induced significant...
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ACC was purified by ammonium sulfate precipitation from freshly excised liver or adipose tissue or from adipose tissue incubated in the absence of hormones prior to homogenization. Samples were incubated (20 min) with the appropriate additions of sodium glutamate (Glu; 100 mM), citrate, and microcystin-LR (M-LR; 1 μM) prior to assay. V_{\text{max}} and K_{\text{m}} were calculated as described (44) using the expression $V = V_{\text{max}} \cdot (C + C_0)$ where $C = \text{citrate concentration (several concentrations, 0–20 mM)}$. Activities (mean ± S.E., determined with at least three tissue preparations) are expressed as percent of the activity with 20 mM citrate. Specific activities of ACC (20 mM citrate) were 12 (liver) and 35 (adipose tissue) milliunits/mg protein.

### Effects of glutamate on kinetic properties of acetyl-CoA carboxylase from rat liver and white adipose tissue

| ACC | Additions to incubation | ACC activity | Kinetic properties of ACC |
|-----|------------------------|--------------|--------------------------|
|     |                        | 0 mM citrate | 20 mM citrate | V_{\text{max}} (mm) | K_{\text{m}} (citrate) |
| A. Liver | − | − | 14.9 ± 1.2 | 100.0 | 108.5 ± 4.0 | 3.3 ± 0.9 |
|         | + | − | 66.8 ± 5.3\textsuperscript{a} | 162.7 ± 5.1\textsuperscript{a} | 191.1 ± 1.5\textsuperscript{a} | 6.6 ± 1.4 |
| Adipose | − | − | 6.2 ± 0.9 | 100.0 | 173.1 ± 35.1 | 16.5 ± 7.3 |
|         | + | − | 36.7 ± 9.9\textsuperscript{a} | 160.2 ± 6.7\textsuperscript{a} | 178.0 ± 9.7 | 1.8 ± 0.6 |
| Adipose, incubated | − | − | 16.8 ± 1.0 | 100.0 | 105.0 ± 1.5 | 2.6 ± 0.3 |
|         | + | − | 62.8 ± 4.7\textsuperscript{a} | 113.0 ± 3.1\textsuperscript{a} | 116.8 ± 1.9\textsuperscript{a} | 2.7 ± 0.8 |
| B. Liver | − | + | 2.8 ± 0.4\textsuperscript{a,b} | 39.7 ± 0.9\textsuperscript{a} | 75.9 ± 9.5 | 20.4 ± 5.5 |
|         | + | + | 12.9 ± 2.4\textsuperscript{a,b} | 63.4 ± 1.4\textsuperscript{a,b} | 95.8 ± 8.2\textsuperscript{b} | 12.8 ± 2.7 |
| Adipose | − | + | 5.7 ± 1.1 | 82.2 ± 3.2\textsuperscript{a} | 171.7 ± 17.3 | 25.1 ± 5.8 |
|         | + | + | 15.4 ± 3.3\textsuperscript{a,b} | 94.7 ± 10.1\textsuperscript{b} | 143.5 ± 9.4 | 10.1 ± 3.1\textsuperscript{a,b} |
| Adipose, incubated | − | + | 13.5 ± 2.0 | 92.8 ± 2.7 | 100.2 ± 2.5 | 3.0 ± 0.6 |
|         | + | + | 44.8 ± 5.5\textsuperscript{a,b} | 99.7 ± 2.3\textsuperscript{b} | 114.4 ± 4.3 | 5.7 ± 1.5 |

\textsuperscript{a} Significant effect of glutamate (p < 0.05).

\textsuperscript{b} Significant effect of microcystin LR (p < 0.05).

### FIG. 2. Effects of glutamate are influenced by the phosphorylation state of ACC. ACC was partially purified from rat liver by ammonium sulfate fractionation (open symbols) or was highly purified by avidin–agarose affinity purification (closed symbols). Preparations were obtained with (dephospho-ACC, squares) or without (phospho-ACC, circles) an incubation step, included after ammonium sulfate treatment (90 min, 37 °C), to allow dephosphorylation by endogenous protein phosphatases. The values shown are from a single experiment that was repeated with very similar results.

ACC activation when no correction was made for ionic strength (Fig. 1) (39, 40) and also when the ionic strength was kept constant by reducing the concentration of sodium chloride or the sodium salt of HEPES (Figs. 2–5).

**Effects of Glutamate Depend on the Extent of ACC Phosphorylation**

The phosphorylation state of ACC significantly influences the sensitivity to citrate and other allosteric ligands. We therefore tested the importance of ACC phosphorylation state in responses to glutamate.

**Purification of “Phospho”-ACC and “Dephospho”-ACC**—We first tested the effects of controlling the extent of ACC dephosphorylation by endogenous protein phosphatases during enzyme purification. Phospho-ACC was prepared, in the presence of phosphatase inhibitors, from rat livers that had been removed and homogenized without freeze-clamping to allow post-mortal phosphorylation of ACC, notably by AMPK (49). Alternatively, dephospho-ACC was prepared using a protocol that included an incubation to allow dephosphorylation, as described under “Experimental Procedures.” Loss of phosphate in this protocol was confirmed as described below. Preparations of phospho-ACC and dephospho-ACC showed dramatically different responses to glutamate following partial purification by ammonium sulfate precipitation or after further purification by affinity chromatography (Fig. 2). Dephospho-ACC preparations exhibited 6–12-fold higher maximum activities in the presence of glutamate and half-maximal activities at glutamate concentrations of 10–15 mM (Fig. 2).

To confirm that phospho-ACC and dephospho-ACC preparations indeed contained different levels of phosphate, preparations were subjected to phosphorylation with AMPK. The incorporation of phosphate into dephospho-ACC (0.8–1.5 mol of phosphate/subunit) exceeded that into phospho-ACC isoforms (0.8–1.5 mol of phosphate/subunit) exceeded that into phospho-ACC isoforms (0.8–1.5 mol of phosphate/subunit).

Proteolysis rather than dephosphorylation might account for ACC activation induced by incubation during purification (50). Two observations suggest this is unlikely. First, SDS-polyacrylamide gel electrophoresis, followed by staining of protein bands and Western blotting with horseradish peroxidase-streptavidin, revealed that there was no significant loss of ACC subunits and Western blotting with horseradish peroxidase-streptavidin, revealed that there was no significant loss of ACC subunits.
maximum activity was 500 milliunits/mg of protein. When purified ACC was co-purifying phosphatase activity. This possibility is unlikely because glutamate is most effective in activating purified ACC malt on highly purified ACC might be explained by residual co-purifying phosphatase activity. This possibility is unlikely because glutamate is most effective in activating purified ACC that is already substantially dephosphorylated. Furthermore, purified ACC preparations contained little or no detectable phosphatase activity. When purified ACC was stoichiometrically phosphorylated with AMPK and [γ-32P]ATP, <5% of the incorporated 32P was removed during subsequent incubation for up to 60 min with glutamate.

Glutamate Promotes Polymerization of ACC

The effects of allosteric regulators and of changes in phosphorylation of ACC are accompanied by parallel changes in catalytic activity and interconversion between inactive dimers and active polymers. We therefore examined the effects of glutamate on the state of polymerization of ACC. Following ammonium sulfate purification from rat liver, ACC was incubated with microcystin LR in the absence or presence of glutamate and subjected to size-exclusion chromatography. Representative chromatograms (Fig. 5) demonstrate that ACC that had been dephosphorylated during purification was largely present as dimers (~500–600 kDa) and that incubation with glutamate (50 mM), prior to and during chromatography, led to a marked shift toward larger molecular size. ACC preparations that had not been subjected to dephosphorylation during purification showed smaller increases in the extent of polymerization induced by glutamate than seen with dephosphorylated ACC. Similar results were obtained using ACC affinity-purified from liver or adipose tissues; and in all cases, the maximal size and proportion of highly polymerized ACC were similar to those seen with maximally activating concentrations of citrate.
The phosphorylation state of ACC plays a crucial role in dictating the sensitivity of the enzyme to glutamate as well as glutamine metabolism. Amino acids provide a carbon source for gluconeogenesis, and fatty acid synthesis. The studies described here confirm earlier reports demonstrating glutamate-stimulated dephosphorylation and activation of hepatic ACC by a type 2A protein phosphatase (39, 40). We also show that glutamate-induced dephosphorylation and activation occur following partial purification and that this property is exhibited by both major ACC isoforms in extracts from rat heart and white adipose tissue. Incubation of adipose tissue preparations in the absence of microcystin LR, unlike corresponding fractions from liver, induced no appreciable ACC activation unless glutamate was also added (Fig. 1 and Table I). There is therefore a significant glutamate-independent (and microcystin-sensitive) ACC phosphatase in fractions from liver, but not from adipose tissue.

The studies presented here demonstrate that glutamate also promotes activation of ACC isoforms by additional, microcystin-insensitive mechanisms. It seems most likely that glutamate acts directly as an allosteric activator of ACC, especially as activation of highly purified ACC is observed under conditions that preclude dephosphorylation. The three-dimensional structures of citrate and glutamate can be aligned very closely, including superposition of two carboxyl groups. This structural overlap suggests that citrate and glutamate might interact with similar residues on ACC. The fact that glutamate and citrate have non-additive effects on maximum ACC activity also suggest similar sites and/or mechanisms of action.

The phosphorylation state of ACC plays a crucial role in dictating the sensitivity of the enzyme to glutamate as well as to citrate and other allosteric ligands. The effects of phosphorylation on glutamate sensitivity are evident when purified ACC is treated with protein kinases or when the phosphorylation state is increased or decreased by incubating intact tissue. AMPK produced the most extensive decline in glutamate sensitivity in vitro, confirming the important role of this protein kinase established using citrate as an allosteric activator (3). The low sensitivity to glutamate of ACC obtained from liver kinase established using citrate as an allosteric activator (3). Significantly, systems that display such characteristics, and multiple-site control also show sharp concentration dependences that provide dramatic responsiveness to small changes in ligand concentration (53).

The sensitivity of ACC to citrate, in absolute terms, is higher than that to glutamate, with $K_c$ values in the range 0.5–10 mM for citrate and 15–30 mM for glutamate. The $K_c$ values might suggest that glutamate is less important as a physiological agonist than citrate. In fact, cellular concentrations of glutamate (5–30 mM) in liver, heart, and fat tissue are substantially higher than those of citrate, which rarely exceed –2.0 mM (54–58). Cellular concentrations of glutamate therefore reach the range that may have an impact on ACC activity. Most likely, the combination of glutamate and citrate serves to tonically activate ACC, the activation being overcome by catabolic and stress signals that activate PKA and/or AMPK. Other organic acids might also contribute to ACC activation, although we found no corresponding effects for a range of potential effectors. In addition to exerting “constitutive” effects, the intracellular concentrations of glutamate might be altered under certain physiological conditions. For example, elevated extracellular concentrations of glutamate can enhance hepatocyte and myocyte glutamine levels and therefore the capacity to generate intracellular glutamate (39, 55). In this context, the control of glutaminase (sensitive to hormones, phosphate, and citrate) might also be crucial (59). Interestingly, glutamine appears to suppress lipolysis and ketogenesis when infused into dogs (60) and exerts cardioprotective effects during ischemia and reperfusion that have been ascribed to enhanced supply of intermediates for the citric acid cycle and thus substrate oxidation (61). In view of our studies, the cardioprotective effects of glutamine might also be partly explained by activation of myocardial ACC and malonyl-CoA production and the subsequent moderation of fatty acid oxidation.

From a general perspective, it is clear that amino acid metabolism interlocks extensively with fatty acid and carbohydrate metabolism. Amino acids provide a carbon source for central pathways such as those involved in energy metabolism, gluconeogenesis, and fatty acid synthesis. The studies described here add to the growing view that amino acids may play important roles as regulatory signals as well as substrates for metabolism (62–64).

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**Table II**

**Effects of glutamate on acetyl-CoA carboxylase polymerization**

ACC was subjected to size-exclusion chromatography (see “Experimental Procedures” and the legend to Fig. 5) in the absence or presence of glutamate (Glu; 50 mM). Four preparations of ACC were analyzed after purification by ammonium sulfate precipitation (ASP) or following further affinity purification (as described under “Experimental Procedures.” The distribution of ACC between dimeric form (fractions 12–20) and polymeric forms (fractions 5–11) was based on ACC activity, measured in all fractions and expressed as percent total recovered activity.

| ACC preparation | Dimeric ACC (400 kDa) | Polymeric ACC (>2000 kDa) |
|-----------------|----------------------|---------------------------|
| Purification    | Phosphorylation       | Glu | +Glu | Glu | +Glu |
| ASP             | Phospho              | 93  | 70   | 7   | 30   |
| ASP             | Dephospho            | 82  | 47   | 18  | 53   |
| ASP             | Dephospho            | 78  | 51   | 22  | 49   |
| Affinity        | Dephospho            | 65  | 47   | 35  | 53   |

(Tables II).
