Activation of Immobilized Acetyl-Coenzyme A Carboxylase

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Partially purified acetyl-CoA carboxylase was covalently bound to a Sepharose 4B matrix. Although aggregation was thus prevented, the enzymic activity was stimulated by citrate and isocitrate.

The transition of acetyl-CoA carboxylase from an inactive protomeric to an active polymeric state is stringently regulated by citrate and isocitrate. The two metabolites stimulate the enzymic activity about 50-fold (Gregolin *et al.*, 1968*a,b*) and simultaneously induce the protomers to rearrange into filaments comprising up to 20 protomeric units (Kleinschmidt *et al.*, 1969). Landman & Dakshinamurti (1975) demonstrated that apo-(acetyl-CoA carboxylase) that lacks enzymic activity is not capable of polymerization. Landman (1976*a*) proposed that the mechanism of aggregation provides the means by which holo-(acetyl-CoA carboxylase) synthetase distinguishes between apo-(acetyl-CoA carboxylase) not capable of polymerization and the respective holoenzyme, which is aggregated by citrate and isocitrate, thus allowing the specificity of biotin binding to be directed towards the apoenzyme. To further explore a possible interrelation between activation and aggregation, the effect of citrate and isocitrate on Sepharose-bound acetyl-CoA carboxylase was examined.

Experimental

Materials

Sepharose 4B was from Pharmacia Fine Chemicals, Uppsala, Sweden. CNBr was from Aldrich Chemical Co., Milwaukee, WI, U.S.A. NaHCO_3 and $\text{NaH}^{14}\text{CO}_3$ (10 mCi/mmol) were from New England Nuclear, Dorval, P.Q., Canada. All other chemicals were from Sigma Chemical Co., St. Louis, MO, U.S.A.

Preparation of gel-bound acetyl-CoA carboxylase

Sepharose 4B was activated essentially as described by Cuatrecasas & Anfinsen (1971). Acetyl-CoA carboxylase was purified by a procedure reported elsewhere (Landman, 1976*b*). Partially purified enzyme (24.2 mg, 0.87 unit/mg) was mixed for 18 h with 8.4 ml of activated Sepharose in 0.1 M-sodium bicarbonate buffer, pH 8.1, containing 0.4 M-NaCl. After the gel slurry had settled, the supernatant was

removed and the gel was washed three times with 0.05 M-Tris buffer, pH 7.5, containing 10 mM- β -mercaptoethanol and 0.1 mM-EDTA. The supernatants were freeze-dried, and then dissolved in a minimal volume of Tris/HCl buffer and assayed.

Enzyme assay

Samples (1.3 ml) of the gel slurry were preincubated in 20 ml vials with an equal volume of assay mixture containing 0.05 M-Tris/HCl, pH 7.4, 0.2 mM-acetyl-CoA, 4 mM-ATP, 8 mM-MgCl₂, 10 mM- β -mercaptoethanol, 0.1 mM-EDTA and 10 mM-sodium citrate, 10 mM-sodium isocitrate or 20 mM-sodium tricarballoylate (propane-1,2,3-tricarboxylate). The reaction vials were shaken in a Dubnoff bath for 15 min. The assay was initiated by adding $\text{NaH}^{14}\text{CO}_3$ (0.1 $\mu\text{Ci}/\mu\text{mol}$) to a final concentration of 20 mM. After 15 min of incubation at 37°C the assay was terminated by adding 1 ml of 6 M-HCl. The gel samples were transferred to Pasteur pipettes. Each column was, subsequently, eluted into a scintillation vial with 15 ml of 1 M-HCl and the eluate incubated at 60°C for 15 min. Each eluate was freeze-dried and 1 ml of water followed by 15 ml of scintillation fluid added to the residue. Samples were counted for radioactivity in a Beckman LS 250 spectrometer.

Protein assay

Protein concentration was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Results and Discussion

At the basic pH and high ionic strength necessary for the binding of ligands to activated Sepharose, acetyl-CoA carboxylase is found essentially in its protomeric form (Gregolin *et al.*, 1968*b*). This is likely to increase the efficiency of enzyme binding to the gel, since there are more enzyme units available for binding when the enzyme is in its protomeric form than when it exists in aggregates. Table 1

Table 1. *Binding of acetyl-CoA carboxylase to activated Sepharose*

Partially purified preparation of acetyl-CoA carboxylase was bound to activated Sepharose 4B. The gel slurry was washed three times and each supernatant was concentrated. The assay procedure was described by Landman & Dakshinamurti (1975).

Fraction	Activity (units)
Acetyl-CoA carboxylase activity added to the gel	21.0
Enzymic activity in the first wash	1.84
Activity in the second wash	0.57
Activity in the third wash	Not detectable

Table 2. *Activation of immobilized acetyl-CoA carboxylase*

Samples of gel-bound acetyl-CoA carboxylase were incubated with a complete assay mixture in presence of 10mM-citrate, 10mM-isocitrate or 20mM-tricarballoylate. The assay procedure is given in the Experimental section. The two columns show the results of duplicate measurements.

Assay system	Radioactivity (c.p.m.)	
Complete mixture with citrate and isocitrate at zero time	476	326
Complete mixture, citrate and isocitrate omitted	643	768
Complete mixture containing tricarballoylate	547	766
Complete mixture containing citrate	1710	1769
Complete mixture containing isocitrate	2019	1894

compares the total enzymic activity added to the gel with that in the wash and shows that about 90% of the activity was bound to the matrix.

The conditions used for the assay of gel-bound acetyl-CoA carboxylase are, obviously, less than optimal, as the bound enzyme and the substrates may be differently distributed inside and outside the beads. The activities obtained in this report can therefore be examined only on a comparative basis. Table 2 shows that tricarballoylate had no apparent effect on matrix-bound acetyl-CoA

carboxylase, whereas citrate and isocitrate activated the enzyme to a different extent. This may be in keeping with a report by Lane and co-workers (Kleinschmidt *et al.*, 1969) that implies that the two activators may affect aggregation in a slightly different mode. Their electron micrographs show different forms for polymers induced by citrate and isocitrate, the former appearing as linear filaments, whereas the latter are branched structures. Sepharose-bound acetyl-CoA carboxylase was activated only 4-fold, much less than 50-fold activation commonly observed with the partially purified free enzyme. This discrepancy may be related to an inherent low activation capability of the bound enzyme or to incubation conditions that are inadequate for optimal activation. However, the possibility of structural damage through the attaching process is not excluded. The finding that citrate and isocitrate activated acetyl-CoA carboxylase, even when aggregation was prevented, substantiates a model proposed by Greenspan & Lowenstein (1968) by which the enzyme may exist in an intermediate form as an active protomer, and further demonstrates that, although aggregation and activation are closely interrelated, they can be uncoupled and examined separately.

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