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Propionyl-CoA Synthetase in Mammary Gland and Liver of Cows

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Propionyl-CoA synthetase of liver and mammary gland from calf and midlactation cow was investigated. No activity of this enzyme was detected in calf mammary gland, but it was detected in calf liver. Propionyl-CoA synthetase was found in both, liver and mammary gland of the cow, although mammary gland activity was about 25 % of that found in liver. The effects of pH and temperature on enzyme activity and stability were also investigated in crude extracts of liver and mammary gland tissues. The results suggest a different behaviour of the enzyme from both origins. Kinetic studies of the enzyme were also carried out, showing differences, depending on the organ, in the apparent substrate K_M values.

Key words: Gluconeogenesis, Mammary Gland, Propionate, Ruminants.

In ruminants, microbial fermentation in the rumen leads to the production of large amounts of short chain fatty acids, mainly acetate, propionate and butyrate. Little dietary hexose is available. This fact implies that glucose has to be synthesized by the gluconeogenic pathway (6, 9). Although acetate and butyrate are the principal lipogenic precursors (1), propionate seems to be the only volatile fatty acid that contributes to gluconeogenesis (15). Estimates of the percentage of glucose arising in this way range from 30 to 50 % (1, 3, 5, 6).

Fatty acid activation constitutes the first step in the metabolism of these compounds, which means the activations involving the formation, by an enzyme reaction, of acyl-CoA derivatives (2). Substrate specificity studies have shown specific acyl-CoA synthetases for acetate, propionate and butyrate (5).

Thus, regulation of propionyl-CoA synthetase plays a key role in control of the gluconeogenic pathway in ruminants.

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Moreover, in special physiological conditions, such as pregnancy and lactation, enough glucose must be synthesized in order to supply the high carbohydrate requirements of these animals (14). If a lactose content in milk is assumed to be 4.6 %, a high producing cow (70 l/d) must synthesize at least 3.22 kg of glucose daily. Considering that about 60 % of the available glucose is used for lactose formation (4), glucose daily requirements would be approximately 5.36 kg. Under these special physiological conditions, a high rate of propionate utilization, as a glucose precursor, seems to be necessary.

Although liver is the main gluconeogenic organ, under these physiological conditions, further synthesis of glucose of other tissues seems to be necessary in order to supply glucose requirements. Whole-body studies in these animals (5, 15) and the occurrence of propionate in the mammary gland artery of the cow (13) suggests the existence of propionyl-CoA synthetase in the mammary gland.

In the present work, a comparative study of propionyl-CoA synthetase from bovine liver and mammary gland has been carried out, in order to achieve further knowledge of the special gluconeogenic mechanism in these animals in relation to milk formation.

Materials and Methods

Liver and mammary glands from several calfs and midlactation cows were obtained from a local abattoir and immediately frozen and kept at −20 °C until used. Minced liver tissues were homogenized (1:4) in an ice-cold solution of 20 mM triethanolamine-HCl, 1 mM GSH and 1 mM EDTA adjusted to pH 7.5. The resulting homogenate was then centrifuged at 25.000 × *g* for 20 min at 5 °C and the supernatant was used as crude extract for enzyme estimation.

The secretory tissue of the mammary gland was separated from the connective by means of dissection, then minced and homogenized in a tissue blender. The homogenate was centrifuged at 25,000 × *g* at 5 °C for 20 min the supernatant being used as crude extract.

Propionyl-CoA synthetase activity was assayed with the slightly modified method of JONES and LIPPMAN (8), based on the spectrophotometric measurement of the ferric complex of propionyl-hydroxamic acid formed by the reaction of the enzymatically produced propionyl-CoA with hydroxilamine. Aliquots of 0.1 ml of each extract was incubated for 30 min at 37 °C with 100 mM Tris-HCl buffer, pH 8.5, 2.5 mM CoA, 10 mM ATP, 20 mM potassium propionate, 200 mM hydroxilamine pH 7.4, 10 mM magnesium chloride and 10 mM GSH in a total volume of 1 ml. The reaction was stopped by adding 1.5 ml of 10 % $FeCl_3 \cdot 6H_2O$ with 3.3 % trichloroacetic acid in HCl 0.66 N. After a 7.000 × *g* centrifugation during 5 min, the propionyl-hydroxamic acid formed was measured at 540 nm. A unit of enzyme (Kat) is the amount of activity that converts one mole of propionate per second at 37 °C. Specific activity is designed as unit per kg of protein (Kat/kg protein).

The effect of pH on enzyme activity was determined by using different buffers with a pH range between 6.0 and 10.0. For pH stability assay, a preliminary 5 min preincubation at 37 °C with different buffers were carried out. In relation to temperature, an incubation temperature range between 0 and 70 °C was used. Temperature stability assays were carried out by a 5 min preincubation of the crude extracts at different temperatures. Activation energy was calculated by using the Arrhenius equation while a Van't Hoff coefficient (Q_{10}) was obtained as increased velocity at different temperatures.

Protein was estimated according to LOWRY *et al.* (10).

Statistical analysis. Results are expressed as the mean ± SD, specifying the number of individual determinations (n).

Statistical means was assessed by the student's «t» test.

Results and Discussion

Propionyl-CoA synthesis can be carried out in bovine liver and mammary gland. However, no activity was detected in calf mammary gland (table I), suggesting that the physiological conditions of these ruminants is a factor to be considered in relation to propionate metabolism in this organ. These findings suggest that one of the functions of the cow mammary gland can be to supplement that of the cow liver gluconeogenesis during lactation, as indicated by specific activity values for the enzyme from liver and mammary gland of adult animals. Enzymatic activity was also considerable in calf liver, indicating an apparent active propionate metabolism in non-lactating ruminants. The occurrence of propionate activation enzymes in the mammary gland was proposed by WILLIAMS and ELLIOT (13) and supported by the presence of propionate in cow mammary gland artery. This suggestion has been corroborated by others (5). The lack of activity of propionyl-CoA synthetase in mammary gland of calf may be explained by insufficient development of the gland or by the lack of the physiological conditions leading to enzyme induction (11). Taking into account that the weight of functional tissue of the midlactation cow mammary gland is higher (about twice) than that of liver, the gluconeogenesis capacity of both organs seems to be similar.

In the present study the effect of propionyl-CoA synthetase activity and stability has been investigated in relation to pH and temperature. As shown in table II, the enzyme from liver and mammary gland of mid-lactation cows exhibited maximal activity and stability in a pH range of 7.5 and 9.0, showing slight differences in relation to pH. Propionyl-CoA synthetase exhibited increasing activity up to 40 and 50 °C from liver and mammary gland tissues respectively.

Table I. *Specific activity of propionyl-CoA synthetase in liver and mammary gland tissues from calf and adult cow.*

The data are expressed as specific activity (μKat/kg protein) ± SD with the number of determinations in parenthesis. n. d. = not detected.

	Liver	Mammary gland
Calf	0.36 ± 0.005 (10)	n. d. (8)
Cow	0.40 ± 0.07 (9)	0.20 ± 0.04 (10)

Table II. *Effect of pH and temperature on activity and stability of the propionyl-CoA synthetase in crude extracts of liver and mammary gland tissues from mid-lactation cows.*

The maximal activity and stability were obtained with 100 mM Tris-HCl as buffer. For activation energy and Van't Hoff coefficient determinations, only activity values at temperatures between 0 and 30 °C were considered, because in this temperature range the enzyme was not denatured.

	Liver	Mammary gland
pH range of maximal activity	7.5-8.5	8.0-9.0
pH range of maximal stability	8.0-9.0	8.5-9.0
Temperature of maximal activity (5 min)	40 °C	50 °C
Activation Energy (kJ/mol)	6.8	2.9
Temperature coefficient (Q_{10})	2.0	1.5
Temperature of maximal stability (5 min)	0-30 °C	0-30 °C

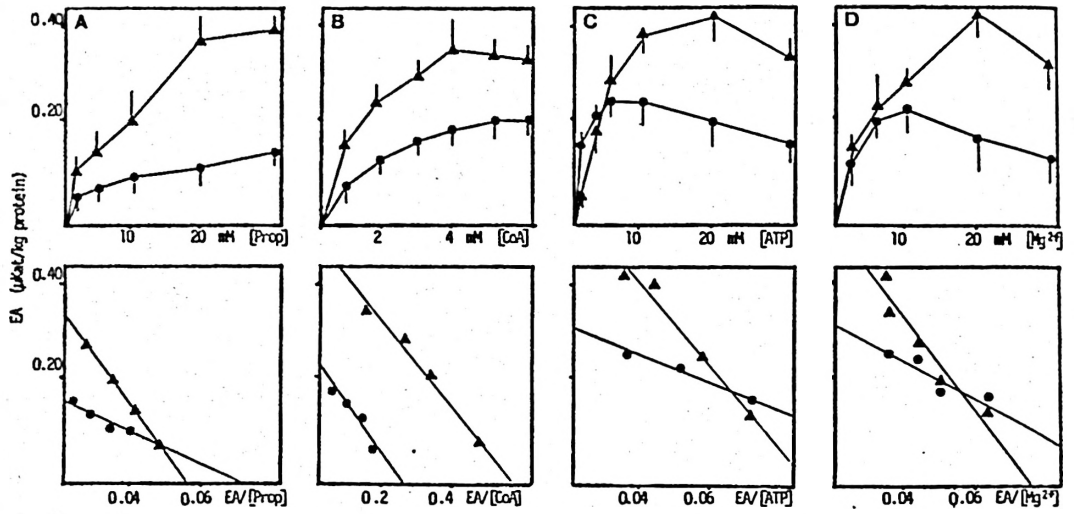

Fig. 1. *Michaelis-Menten plot (upper) and Eadie-Hofstee plot (lower) for the different substrates, coenzymes and cofactors of propionyl-CoA synthetase from liver (▲) and mammary gland (●).* The enzyme assays were carried out as decribed in Methods section, except varying the concentration of propionate (A), coenzyme A (B), ATP (C) and Mg^{2+} (D). Specific activities are means ± SD for three to six different determinations.

Furthermore, the enzyme from both organs was stable at pH 8.5 for 20 min up to 30 °C. At higher temperatures the enzyme became inactivated. However, in relation with the temperature dependent activity, the calculated activation energy appears to vary depending on the organ from which it originated. Moreover, the temperature coefficients (Q_{10}) were also different. These results suggest that in mid-lactation cow propionyl-CoA synthetase from liver and mammary gland is under the influence of different regulatory mechanisms.

The K_M values for substrates, coenzymes and cofactors involved in the reaction catalyzed by the propionyl-CoA synthetase from liver and mammary gland of mid-lactation cows are shown in table III. Propionate affinity for the liver enzyme is clearly lower than the mammary gland. The K_M of propionate from liver propionyl-CoA synthetase was higher than the value reported previously (12). However, the Michaelis-Menten plot in figure 1, where the maximum velocity is rather difficult to calculate, takes into account the fact that at 30 mM propionate no substrate saturation seems to occur, whether the propionyl-CoA synthetase is from liver or from mammary gland. A similar observation, reported elsewhere (12), indicated that at propionate concentrations higher than the physiological ones, the *in vitro* assay results are somewhat confusing, which could be explained from the assumption that in the crude extract contents also, another acyl-CoA synthetase will function at high propionate concentrations thus preventing saturation. The absence of propionate saturation in the Michaelis-Menten plot (fig. 1 A upper) was overcome by using the Eadie-Hofstee plot (fig. 1 A lower) where the saturating concentration and the apparent K_M value were easily calculated.

Table III. *Michaelis constant (K_M) values (mM) for the substrates, coenzymes and cofactors of propionyl-CoA synthetase in crude extracts of liver and mammary gland tissues of the mid-lactation cow.* Apparent K_M values were calculated for Eadie-Hofstee plots and determined using different substrate concentrations in the assay conditions.

	Liver	Mammary gland
Propionate	5.3	1.3
Coenzyme A	0.9	1.0
ATP	4.8	1.1
Mg^{2+}	5.5	1.6

The apparent propionate K_M for mammary gland propionyl-CoA synthetase was lower than the one for liver, probably due to lower availability of propionate in mammary gland artery, being quite similar to that from bovine liver mitochondria and dairy cow liver (12), as well as to values found for guinea-pig liver mitochondrial enzyme (7).

With ATP as substrate and Mg^{2+} as cofactor, the difference between K_M values for the enzyme from both origins was marked (fig. 1). Moreover, propionyl-CoA synthetase from both organs was inhibited by high substrate concentrations. The inhibition of liver and mammary gland enzyme appear when substrate was above 20 and 10 mM respectively. The high ATP K_M value described by us for the liver enzyme as compared with the results of RICKS and COOK (12), may be due to the presence in the liver crude extract of ATPase activity, which may decrease ATP concentration in the assay mixture.

The K_M CoA values for the liver and mammary gland enzymes are similar, and agree with those reported for the mitochondrial liver enzyme from lactating cow (12) and guinea-pig (7).

Finally, as shown in table IV, the propionate-activating enzyme from cow was assayed by using the same concentration of acetate, propionate and butyrate. The enzyme showed activity with all these substrates, but propionate produced the highest activity. It should also be noted, that less activity was observed when sodium salts were used instead of potassium, implying a possible inhibition effect of sodium ion on this reaction or lack of activation by K^+.

The enzyme from both organs has apparently a preferential specificity for propionate, therefore being operative in gluconeogenesis. The presence of propionyl-CoA synthetase in female lactating bovine mammary gland, in spite of the different kinetic behaviour respect to the liver enzyme, could imply the participation of mammary gland in the gluconeogenesis pathway of the adult animal.

Table IV. *Comparative specific activity (μKat/kg protein) of propionyl-CoA synthetase using acetate, propionate and butyrate as substrates.*
The concentration for all substrates was 20 mM. Specific activities were determined using different substrates in the assay conditions. Results are means ± SD for five different determinations. Statistically significant differences: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

	Liver	Mammary gland
Potassium propionate	0.40 ± 0.08	0.17 ± 0.03
Sodium propionate	0.34 ± 0.1	0.11 ± 0.01**
Potassium acetate	0.18 ± 0.08**	0.12 ± 0.03*
Sodium acetate	0.12 ± 0.03***	0.11 ± 0.03*
Potassium butyrate	0.33 ± 0.1	0.10 ± 0.01**
Sodium butyrate	0.28 ± 0.06*	0.07 ± 0.01***

Resumen

Se estudia la propionil-CoA sintetasa de hígado y glándula mamaria de ternera y vaca en estado de producción láctea. En ternera, se detecta actividad enzimática en hígado, pero no en glándula mamaria. Por el contrario, en el animal en estado de producción, se aprecia actividad propionil-CoA sintetasa en los dos órganos en estudio, siendo la actividad en glándula mamaria un 25 % de la encontrada en hígado. Este estudio abarca el análisis físico-químico y cinético de la enzima en ambas localizaciones. En relación al efecto del pH y la temperatura sobre la actividad y la estabilidad enzimática, se observan diferencias dependiendo del órgano. Además, la enzima de ambas procedencias presenta diferencias en cuanto a los valores de K_M obtenidos en relación a sus sustratos y cofactores enzimáticos.

Palabras clave: Gluconeogénesis, Glándula mamaria, Propionato, Rumiantes.

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