SUBSTRATE SPECIFICITY OF FATTY ACID SYNTHETASE FROM YEAST

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1. Introduction

The purified multienzyme complex fatty acid synthetase from yeast synthesizes palmitoyl- and stearoyl-CoA with NADPH, acetyl-CoA and malonyl-CoA as substrates. At least seven different enzymatic steps have been shown to occur in the overall synthesis by the use of appropriate model substrates [1], e.g. S-acetoacetyl-*N*-acetylcysteamine for the β-ketoreductase partial activity. As reported in this communication, the model compounds S-acetyl-*N*-acetylcysteamine and S-malonyl-*N*-acylcysteamine can replace the natural substrates acetyl-CoA and malonyl-CoA in the overall fatty acid synthesis.

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2. Materials and methods

The purification and assay of fatty acid synthetase followed procedures described in [2].

CoASH and NADPH were obtained from Boehringer, Mannheim.

Acetyl-CoA and malonyl-CoA were prepared according to [3, 4], respectively. S-acetyl-*N*-acetylcysteamine was prepared according to [5] and was a gift of Dr. Eggerer. S-malonyl-*N*-capryloylcysteamine and *N*-acetylcysteamine were synthesized according to [4, 6], respectively. S-[1-^{14}C] acetyl-*N*-acetylcysteamine was prepared by treatment of *N*-acetylcysteamine with [1-^{14}C] acetic anhydride (The Radiochemical Centre, Amersham).

Fatty acid analysis by radio gas chromatography was performed as described in [7].

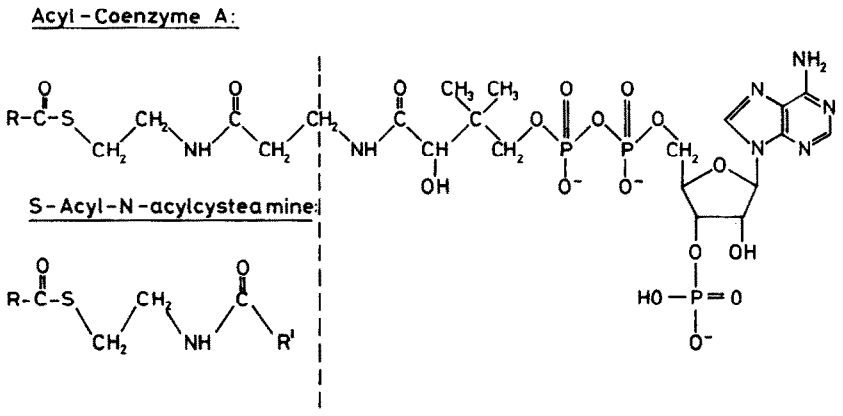

Fig. 1. Chemical structures of the natural substrates and the model substrates of fatty acid synthetase.

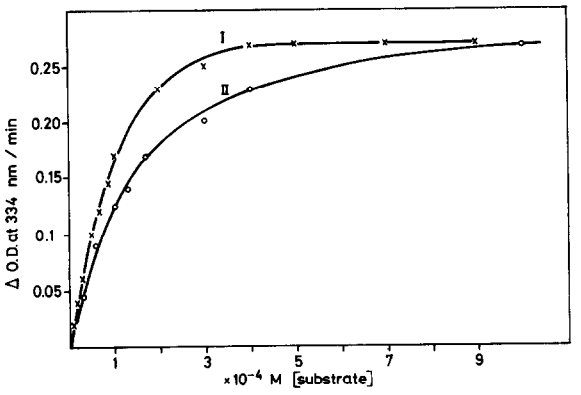

Fig. 2. Fatty acid synthetase activity as a function of the concentration of S-malonyl-*N*-capryloylcysteamine (I) and S-acetyl-*N*-acetylcysteamine (II), respectively. Each of the samples contained in a volume of 1.0 ml: 100 μmole potassium phosphate, pH 7.5; 0.4 mg bovine serum albumin; 0.2 μmole NADPH; 12 μg fatty acid synthetase (2500 mE/mg). Curve I: S-acetyl-*N*-acetylcysteamine, 3 μmole; S-malonyl-*N*-capryloylcysteamine as indicated. Curve II: S-malonyl-*N*-capryloylcysteamine, 1 μmole; S-acetyl-*N*-acetylcysteamine as indicated. The rate of NADPH consumption was measured at 25°.

3. Results and discussion

The model substrates used in this study, S-acetyl-*N*-acetylcysteamine and S-malonyl-*N*-capryloylcysteamine represent structural elements of acetyl-CoA and malonyl-CoA, respectively (fig. 1). Under conditions of enzyme saturation about 70% of the maximum rate of fatty acid synthesis was observed, when S-acetyl-*N*-acetylcysteamine and S-malonyl-*N*-capryloylcysteamine were used in place of the natural substrates. The affinities of the model substrates were found to be smaller than those of the natural substrates. For saturation of the enzyme concentrations about ten times higher were required as compared to acetyl- and malonyl-CoA (fig. 2).

Controls for the actual synthesis of long chain fatty acids were run using S-[1-^{14}C] acetyl-*N*-acetylcysteamine. The analysis of the end products by radio gas chromatography confirmed the synthesis of palmitic and stearic acid (fig. 3).

This work shows that S-acetyl-*N*-acetylcysteamine and S-malonyl-*N*-acylcysteamine are the simplest thiolester derivatives of acetic and malonic acid, which are accepted as substrates by the fatty acid synthetase. More complex model compounds that approach the natural substrates in structure and size, like S-acetylpantetheine and S-malonylpantetheine proved to be substrates as well [8].

References

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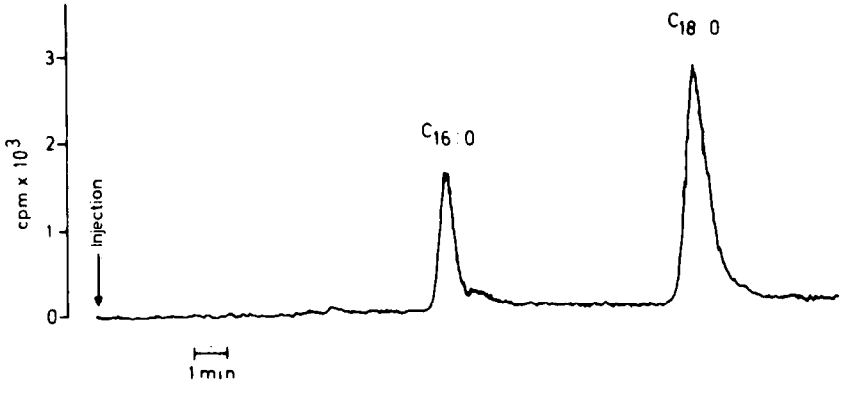

Fig. 3. Radio gas chromatographs of fatty acid methyl esters isolated from incubation mixtures with fatty acid synthetase and model substrates. The incubation mixture (2 ml) contained: 200 μmole potassium phosphate, pH 6.5; 0.8 mg bovine serum albumin; 1.2 μmole NADPH; S-[1-^{14}C]-acetyl-*N*-acetylcysteamine (15 μCi/μmole), 1 μmole; 200 μg fatty acid synthetase (1500 mE/mg); 2 μmole S-malonyl-*N*-capryloylcysteamine. Incubation at 25° for 5 min. The synthesized fatty acids were extracted and analyzed as described in [7].

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