ACYL-CoA: 6-APA ACYLTRANSFERASE FROM *PENICILLIUM CHRYSOGENUM*

STUDIES ON ITS HYDROLYTIC ACTIVITY

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Acyl-CoA: 6-APA acyltransferase (AT) from *Penicillium chrysogenum* Wis 54-1255 catalyzes the hydrolysis of different acyl-CoA derivatives generating, in the absence of 6-APA, free acid and CoA. The hydrolytic efficiency of AT is highest for acyl-CoA variants in which the acyl-moiety is higher than six carbon atoms. The maximal rate of catalysis was achieved in 50 mM Tris-HCl buffer, pH 8.5 at 35°C. Unlike the AT activity, the acylase activity has a different optimum temperature and substrate specificity and dithiothreitol is not required for the reaction.

Acyl-CoA: 6-APA acyltransferase (AT) from *Penicillium chrysogenum* catalyzes the *N*-acylation of 6-APA with the acyl group of several acyl-CoA variants (hexanoyl-, 3-hexenoyl-, heptanoyl-, octanoyl-, phenylacetyl- and phenoxyacetyl-CoA) generating different penicillins (DF, F, H, K, G and V, respectively)[1]. The substrate specificity of this enzyme and its catalytic behavior as AT has been reported in previous papers[2~4]. However, the transferase activity is only one of four activities attributable to a single thiol-dependent enzyme (AT) that functions in a ping-pong mechanism with an alternate hydrolytic step[5]. Study of such hydrolytic activity could be important to establish whether the substrate specificity of AT, and therefore the biosynthesis of penicillins, depends or not on the selectivity imposed by the hydrolytic step.

Materials and Methods

Materials

Acyl-CoA derivatives were obtained from Sigma Chemical Co. (U.S.A.).

Microorganisms

The strain of *P. chrysogenum* Wis 54-1255 used in the experimental work was from the American Type Culture Collection (ATCC No. 28089). The fungus was grown and cultured as previously described[6].

Purification of Acyl-CoA: 6-APA AT

The enzyme was purified as previously reported[3].

Measurement of Hydrolytic Activity

At a total volume of 100 μl the reaction mixture contained the following: 50 mM, Tris-HCl buffer pH 8.5, 80 μl; 10 mM acyl-CoA derivative, 10 μl and pure AT 10 μl (4 μg of protein). Incubations were carried out at 35°C for 20 minutes and halted by adding 100 μl of pure methanol. When required, the buffer, the pH or temperature were modified. The reactions were followed by HPLC using a Spectra-Physics model SP 8800 chromatograph equipped with a variable wavelength UV/VIS detector (SP 8450), a computing integrator (SP 4290) and a microparticulate reversed phase (RP-8) column (Spheri-5, 220 × 4.6 mm i.d.) (Brownlee Laboratories).

The mobile phase used for the separation of the different acyl-CoA derivatives was 0.2 M KH_2PO_4 (P) pH 4.5-2-propanol (IPL). The proportions of P and IPL, the flow rate (Fr) and the retention times (Rt) for each compound were: (1) acetyl-CoA: P-IPL (96.5:3.5); Fr: 1.5 ml/minute; Rt: 5.8 minutes, (2) butyryl-CoA: P-IPL (90:10); Fr: 1 ml/minute; Rt: 7.0 minutes, (3) valeryl-CoA: P-IPL (87:13); Fr: 1.5 ml/minute; Rt: 6.2 minutes, (4) hexanoyl-CoA: P-IPL (75:25); Fr: 1 ml/minute; Rt: 6.3 minutes, (5) heptanoyl-CoA: P-IPL (72:28); Fr: 1 ml/minute; Rt: 6.9 minutes, (6) octanoyl-CoA: P-IPL (70:30); Fr: 1 ml/minute; Rt: 7.0 minutes, (7) nonanoyl-CoA: P-IPL (65:35); Fr: 1 ml/minute; Rt: 4.7 minutes, (8) decanoyl-CoA: P-IPL (65:35); Fr: 1 ml/minute; Rt: 6.5 minutes, (9) phenylacetyl-CoA: P-IPL (90:10); Fr: 1.5 ml/minute; Rt: 8.0 minutes.

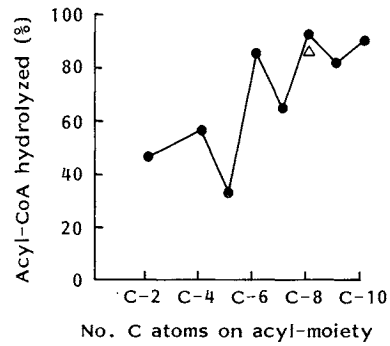
Results and Discussion

Acyl-CoA: 6-APA AT of *P. chrysogenum* is an enzyme that catalyzes the transference of the acyl-moiety of different acyl-CoA variants to the amino group of 6-APA generating different penicillins[1]. This reaction involves a first hydrolytic step in which the acyl molecule is broken, followed by a second one in which the *N*-acylation of 6-APA occurs[3~5]. In order to know whether the substrate specificity of AT might be governed by the specificity of the hydrolytic activity or not, the hydrolytic behavior of this enzyme was investigated.

AT hydrolyzes acyl-CoA variants (hexanoyl-CoA, heptanoyl-CoA and octanoyl-CoA) with an acyl-moiety that can be incorporated as a penicillin side chain precursor[2] and others that cannot be used by this enzyme to acylate 6-APA (acetyl-CoA, butyryl-CoA, valeryl-CoA, nonanoyl-CoA, decanoyl-CoA)[2] (see Fig. 1). The rate of hydrolysis is maximal when the carbon length of the acyl-derivative ranges around 8 carbon atoms (see Fig. 1). Moreover, the rate of hydrolysis is higher when

Fig. 1. Study of the hydrolytic activity of AT against different acyl-CoA variants.

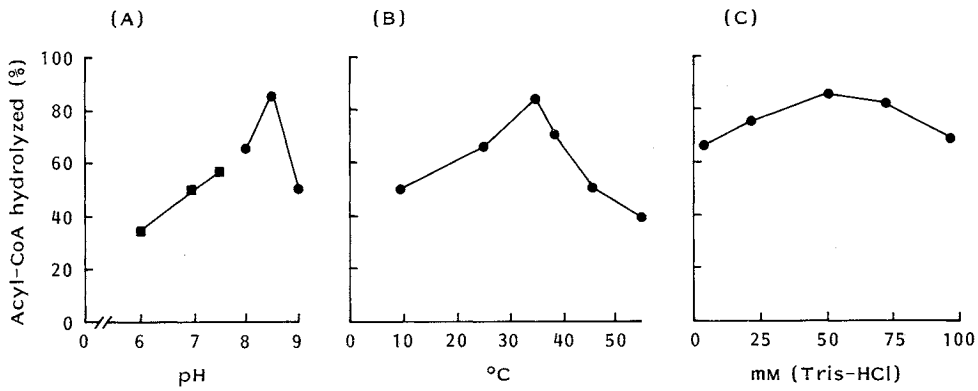
Number of carbon atoms on the acyl-moiety (●), phenylacetyl-CoA (△).



Each compound (1 mM) was incubated with AT at 35°C for 20 minutes.

Fig. 2. Effect of pH, temperature and ionic strength on the hydrolytic activity of AT.

(A) pH, (B) temperature, (C) ionic strength. ■ Phosphate buffer (50 mM), ● Tris-HCl buffer (50 mM).



Phenylacetyl-CoA (1 mM) was incubated with AT at 35°C for 20 minutes.

an even number of carbon atoms is present in the acyl moiety (see Fig. 1). The effect of 6-APA on hydrolytic activity was also investigated by measuring the catalytic rate when this molecule was supplied or not to the reactions. In no case was hydrolysis affected, suggesting that 6-APA is not an effector for the hydrolytic activity of AT (data not shown). Furthermore, dithiothreitol, which is necessary for the "*in vitro*" biosynthesis of penicillins[2~3], is not required for the hydrolysis of these acyl-CoA variants. Additionally, study of the optimal physico-chemical parameters indicates that hydrolysis rate was maximal at pH 8.5 (a pH value similar to that shown by the acyltransferase activity, 8.4) whereas the optimal temperature (35°C) is quite different from that reported for the other activity (20~23°C)[2] (see Figs. 2A and 2B). These data suggest that: a) the substrate specificity of AT working as hydrolase or as AT is quite different; b) the physico-chemical conditions for obtaining the maximal catalytic rate are also different, and c) the fact that 6-APA does not affect the hydrolytic activity indicate that the active site for hydrolysis and 6-APA *N*-acylation are not the same.

In the light of the above results it could be speculated that this enzyme; which catalyzes the last step of the biosynthesis of semisynthetic and natural penicillins, might also play other physiological roles. It is possible that this enzyme might act as an esterase involved in the hydrolysis of CoA derivatives.

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