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Monohydroxamic acid biosynthesis

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Abstract

Hydroxamic acids (HA), with the general formula R–CO–NHOH, are chelating agents which may be used in a number of interesting applications, such as medicine and waste water treatment. In this paper, we describe the enzymatic synthesis of HA with various chain lengths (from C2 to C18), using three microbial enzymes. For short- and middle-chain HA synthesis, using amidases from *Rhodococcus* sp. R312, the optimal working pH was found to be pH 7 or 8, depending on the amide substrate used. Different Michaelis–Menten constants were also determined. For fatty HA synthesis, using lipase from *Candida parapsilosis*, the optimal working conditions were determined to be pH 6, 1 M hydroxylamine and 40°C. Because of the favorable bioconversion yields achieved, the enzymatic synthesis of HA with the appropriate biocatalysts appears to be an interesting alternative to chemical synthesis.

Keywords: Hydroxamic acids; Lipase; Amidase; Hydroxylamine; Aminolysis; Bioconversion

1. Introduction

Hydroxamic acids (HA), with the general formula R–CO–NHOH, form very stable chelates with a number of metal ions. In addition, HA can be found as constituents in such interesting compounds as growth factors, food additives, antibiotics, tumour inhibitors, antifungal agents, cell division factors and enzyme inhibitors [1,2]. The properties of HA are influenced by the nature of their acyl group, notably the carbon chain length. Short-chain HA (C2–C3) are highly water soluble, but some HA, such as acrylohydroxamic acid, can be used as synthons for the production of resins with high chelating properties; middle-chain HA (C4–C8), particularly α-aminohydroxamic acids, are useful for medical applications since they have been described as inhibitors of metalloproteases. Long-chain HA ($>$ C12) are not water soluble, which presents many interesting possibilities, for instance, for their recovery after the extraction of toxic metals from an aqueous medium.

Microbial enzymes isolated in our laboratory have been shown to efficiently catalyse HA synthesis in mild physico-chemical conditions, in reaction media devoid of organic solvents. Different enzymes can be used according to the carbon-chain length of the substrate, which can

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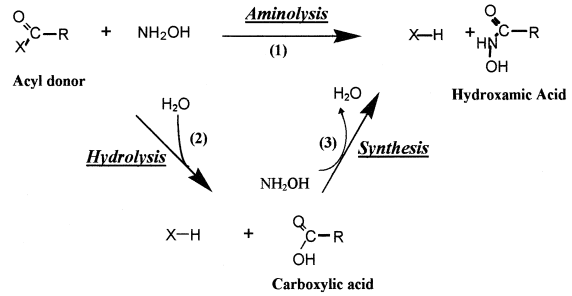

Fig. 1. Possible reactions for the biosynthesis of hydroxamic acids.

be an acid, an ester or an amide. The wide-spectrum amidase from *Rhodococcus* sp. R312 can synthesise short-chain (C2–C3) HA [3], whereas the adipamidase from this bacterium catalyses the synthesis of middle-chain (C4–C8) HA. Hydrophobic long-chain HA are produced in the presence of the lipase/acyltransferase from *Candida parapsilosis* [1]. The present paper describes the influence of various physico-chemical parameters on HA synthesis in the presence of these acyltransferases.

2. Experimental

2.1. Reaction media

2.1.1. Short-chain HA synthesis

Enzyme: wide spectrum amidase from *Rhodococcus* sp. R312

Acyl donors: short-chain amides

Conditions: aqueous solutions of amide and hydroxylamine in the appropriate phosphate buffer (pH 7 or 8), at 30°C.

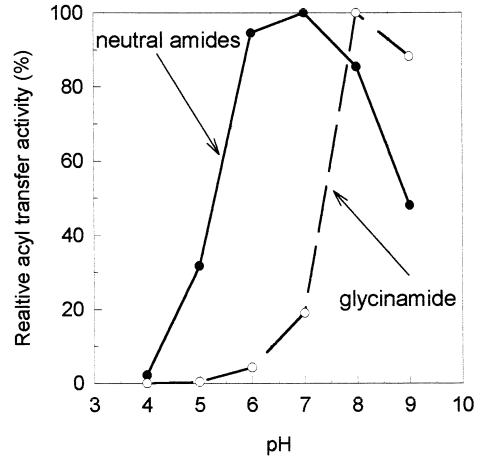

Fig. 2. Influence of pH on the short-chain HA synthesis.

2.1.2. Middle-chain HA synthesis

Enzyme: adipamidase from *Rhodococcus* sp. R312

Acyl donors: middle-chain amides

Conditions: aqueous solutions of amide and hydroxylamine in the appropriate phosphate buffer (pH 7 or 8), at 30°C.

2.1.3. Fatty HA (FHA)

Enzyme: lipase–acyl transferase from *Candida parapsilosis*

Acyl donors: fatty acid ethyl esters

Conditions: stirred aqueous biphasic medium, with emulsified esters and hydroxylamine aqueous solutions at 45°C.

Table 1
Different Michaelis–Menten constants determined for the short-chain HA synthesis by the wide spectrum amidase from *Rhodococcus* sp.R312

Acyl donor	V_m (μmol min^{-1} mg^{-1})	K_{amide} (mM)	$K_{hydroxylamine}$ (mM)	pH
acetamide	948	9	96	7
propionamide	218	22	262	7
acrylamide	246	34	25	7
butyramide	13	620	not determined	7
isobutyramide	9	276	not determined	7
glycinamide	152	421	415	8

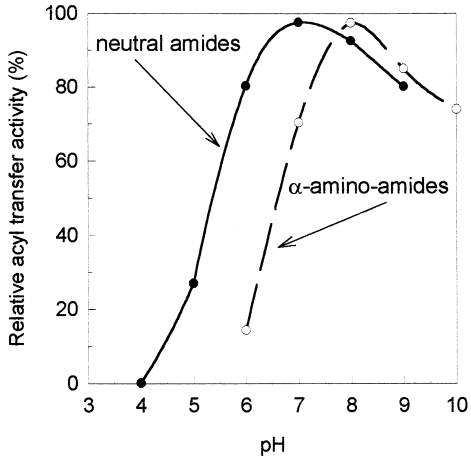

Fig. 3. Influence of pH on the middle-chain HA synthesis.

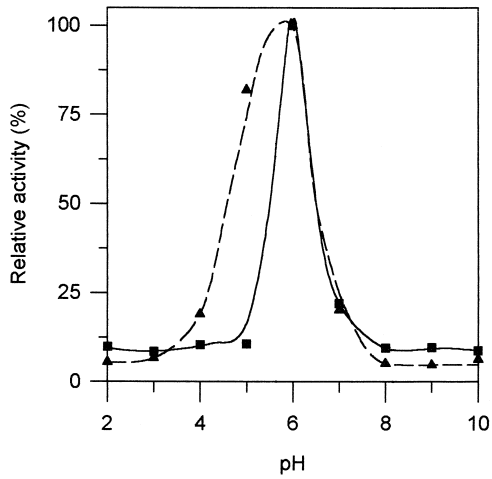

Fig. 4. Influence of pH on fatty HA synthesis (–■–) and on residual hydrolysis (–▲–).

2.2. HA assay

HA were assayed using colorimetric methods with iron(III) [4,5].

3. Reactions involved in the HA production

The acyltransferases used for the HA synthesis are a function of the acyl chain length of the acyl donor, but they all catalyse the acyl transfer reaction from an acyl donor (amide or fatty acid ethyl ester) onto an acyl acceptor (water or hydroxylamine).

The different reactions involved are described in Fig. 1. The amidase-catalyzed reactions are of 'bi-bi-ping-pong' type, and it was shown that the hydrolysis reaction (2), in competition with the direct acyl transfer on hydroxylamine (reaction (1)), was generally very low when sufficient hydroxylamine concentrations were used [3].

The lipase from *C. parapsilosis* is able to catalyse the production of FHA from both fatty esters and fatty acids, but it was also shown that the direct acyl transfer on hydroxylamine was the most efficient reaction [6].

4. Influence of reaction conditions on HA biosynthesis

4.1. Short-chain HA

Short-chain HA were synthesized using the wide spectrum amidase from *Rhodococcus* sp.

Table 2
Different Michaelis–Menten constants determined for the middle-chain HA synthesis by the adipamidase from *Rhodococcus* sp. R312

Acyl donor	V_m (μmol min^{-1} mg^{-1})	K_{amide} (mM)	$K_{hydroxylamine}$ (mM)	pH
butyramide	229	0.1	178	7
isobutyramide	107	0.1	132	7
valeramide	313	0.1	230	7
hexanoamide	335	0.1	549	7
DL-methioninamide	163	12	105	8
L-leucinamide	78	4	68	8
L-alaninamide	66	12	25	8
L-threoninamide	38	16	25	8

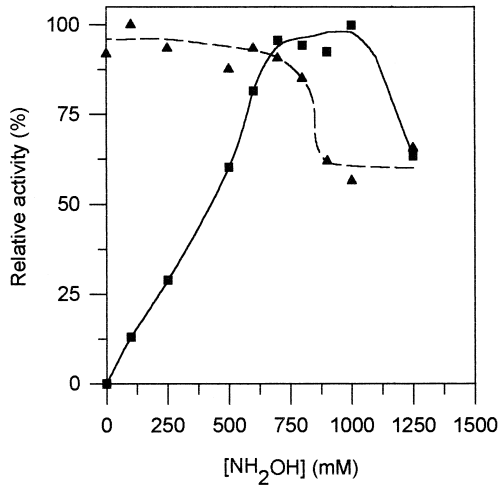

Fig. 5. Effect of hydroxylamine concentration on fatty HA synthesis (–■–) and on residual hydrolysis(–▲–).

R312 overproduced in an *Escherichia coli* strain. As shown in Fig. 2, the optimum working pH was a function of the acyl donor used: pH 7 for all neutral amides and pH 8 for glycinamide. In these conditions, the different Michaelis–Menten constants have been determined and are shown in Table 1. Only very short-chain amides were efficient acyl donors, with both high V_{max} values and low K_{amide} values.

4.2. Middle-chain HA

Middle-chain HA were synthesized using the adipamidase from *Rhodococcus* sp. R312, also overproduced in an *E. coli* strain. As shown in Fig. 3, the optimum working pH was a function of the acyl donor used: pH 7 for all neutral amides and pH 8 for all α-amino amides. In these conditions, the different Michaelis–Menten constants have been determined and are shown in Table 2. Hydrophobic amides turned out to be very good acylating agents for the enzyme (very low K_{amide} values) but the deacylation step with hydroxylamine was easier with α-aminoacyl-enzyme complexes (lower $K_{hydroxylamine}$ values).

4.3. Fatty HA (FHA)

Contrary to the two types of HA mentioned above, the reaction medium was biphasic. The Michaelis–Menten kinetic constants could not be calculated because fatty acid ethyl esters and FHA are not water-soluble. The results concerning the effects of pH and hydroxylamine concentration on FHA synthesis and residual hydrolysis are presented in Fig. 4 and Fig. 5. The optimum working pH for both hydrolysis and FHA synthesis activities was pH 6, but the pH range suitable for the FHA synthesis was narrow. At pH 5, residual hydrolysis and FHA-synthesis activities were 80% and 10% of the respective maximums. The highest FHA production was obtained with 0.7–1.0 M hydroxylamine. Higher concentrations were inhibitory. In the presence of 1 M hydroxylamine, FHA synthesis was maximum and hydrolysis activity was reduced to about 60% of its maximum.

Different temperatures between 22 to 60°C were also tested. The optimum temperature for both hydrolysis and FHA synthesis reactions was found to be 40°C.

Table 3
Bioconversion yields determined for the biosynthesis of various HA

Product	Acyl donor	Hydroxylamine	Enzyme	Reaction time	Molar conversion yields (%)
acetohydroxamic acid	acetamide 100 mM	500 mM	wide spectrum amidase 0.04 g l^{-1}	30 min	86
propionohydroxamic acid	propionamide 100 mM	800 mM	wide spectrum amidase 0.08 g l^{-1}	40 min	57
acrylohydroxamic acid	acrylamide 100 mM	300 mM	wide spectrum amidase 0.08 g l^{-1}	30 min	68
oleylhydroxamic acid	oleic acid ethyl ester	1000 mM	lipase	20 h	40
linoleylhydroxamic acid	linoleic acid ethyl ester	1000 mM	lipase	20 h	40

5. Bioconversion yields

Several HA were synthesized using the wide spectrum amidase from *Rhodococcus* sp. R312 and the lipase from *C. parapsilosis*. Results are shown in Table 3. Bioconversion yields were very good for short-chain HA synthesis and reaction times were short. Bioconversion yields obtained with lipase as a biocatalyst were a little lower and reaction times were longer. But in all cases, interesting HA production was observed.

6. Conclusion

The use of several specific biocatalysts facilitates the synthesis of a wide range of HA. Mild reaction conditions (pH 7–8, temperature below 50°C, aqueous media, biological catalysts) and good bioconversions yields make biological synthesis an interesting alternative to chemical synthesis (which involve very basic or very acid media, high temperatures, sometimes dangerous chemical catalysts and a nitrogen atmosphere). As a follow-up, we intend to investigate the immobilization of the biocatalysts described in this paper in order to study the continuous production of HA in a bioreactor.

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