Enzymology and regulation of the atropine metabolism in pseudomonas putida

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CHAPTER 1

OVERVIEW OF LITERATURE

The breakdown of atropine in mammalians, plants and bacteria has been studied quite intensively as far it concerns the hydrolysis in tropic acid and tropine. The atropine esterase has been shown to be involved in various organisms. In addition, in higher animals the conversion of atropine into some other metabolites has been demonstrated. Since these data have only limited relevance for the research presented in this thesis, only the most interesting data have been included in this overview.

1.1 THE BREAKDOWN OF ATROPINE IN MAMMALIANS

The first indications regarding the metabolism of atropine date from the year 1852 when Schroff, a physician in Vienna, noticed a relative insensitivity of rabbits, which fed themselves with the leaves of the belladonna plant. Rabbits elsewhere in Europe did not show this insensitivity. Nowadays these regional differences are not observed anymore. During the First World War various rabbit varieties in Europe have been mixed (Quinton 1966).

Fleischmannn (1910) and Metzner (1912) were the first scientists to relate the resistance against atropine and the ability of rabbit serum to inactivate atropine. Bernheim (1938, 1948), Glick (1940) and Ammon (1949) proved this inactivation to be caused by enzymatic hydrolysis of atropine in the pharmacological low active hydrolysis products tropic acid and tropine. The enzyme involved was named atropine esterase.

Resistance against atropine has a genetic basis and is transferred as a recessive feature (Sawin and Glick 1943). Recently, Werner (1967) has listed the mammalians that possess this enzyme. In addition to some rabbits, several guinea pigs are atropine resistant and possess the atropine esterase. The enzyme has not been observed in other mammalians or in humans.

Margolis and Feigelson (163, 1964) have made an extensive study of atropine esterase in rabbit serum. These investigators succeeded to purify the enzyme 70 times compared to the starting material and to prepare an antiserum against this atropine esterase for immune titrations using serum of atropine resistant and atropine sensitive rabbits. They concluded that the lack of atropine esterase activity was caused by the absence of the enzyme or an immunological

related protein and not by the presence of an activator of the enzyme.

Not only hydrolysis of tropine-esters, the hydrolysis of esters of choline and glycerol is accelerated as well. Therefore, this atropine esterase is a non-specific esterase. In contrast to the a-specificity is the remarkable preference of the enzyme for the (-) enantiomer of atropine and scopolamine[#]; (-) atropine is hydrolyzed 100 times faster compared with (+) atropine (Werner 1967). The enzyme has no preference for either of the optical isomers of homatropine.

Concerning atropine-like compounds one could add the following. For this atropine esterase only compounds with a 3' substituent of tropine and the N-methyl group in the trans position are suitable substrates. Therefore, Werner (1967) has proposed as the official name for this enzyme: trans (-) hyoscyamine-acyl hydrolase. The cis-isomer of atropine can be hydrolyzed by the serum of all investigated species including by that of human. The atropine esterase is inhibited by organophosphorus compounds di-ethyl-p-nitrophenyl phosphate (Margolis and Feigelson, 1964) and dïisopropyl phosphofluoridate (Berends, 1965 and Otorii, 1965).

The enzymatic hydrolysis of the ester atropine is not the only mechanism for detoxification of atropine in resistant animals. Also the metabolism and excretion of atropine in esterase deficient mammalians has been studied.

Kalser (1957), and Gabourel and Gosselin (1958) have applied radioactive atropine. Following intravenous application, the radioactivity was mainly recovered from urine, partially as atropine, partially conjugated to glucuronic acid.

In this way they confirmed earlier observations (Bernheim 1948) on the unaltered secretion by the kidney. A part of the radioactivity in the urine showed a positive reaction with reagents for the phenyl hydroxyl group: this points to a hydroxylation of the tropic acid residue in atropine. Only 0.3% of the radioactivity was excreted as tropic acid. Using paper chromatography, a number of non-identified products were found. Werner (1968) confirmed the hydroxylation of atropine and found evidence for an oxidative demethylation of the N-methyl group of atropine.

The conversion of atropine in rat liver has been studied by Matsuda (1966). This author compared the pharmacological action spectrum of intravenously applied atropine with that of intravenously applied atropanal (the tropine-ester

chemical formulas are presented in table 5.6 and in annex 1

of 2-phenylmalonic semi-aldehyde). Atropanal appeared to be 7x more toxic but was less active in parasympathetic activity. Atropine slowly applied in the portal vein gives the pharmacological action spectrum of intravenously applied atropanal. Matsuda concluded that atropine is oxidized to atropanal in rat liver.

1.2 THE BREAKDOWN OF ATROPINE IN PLANTS

The members of the plant family of *Solanaceae* are the natural producers of the tropa-alkaloids that include atropine, scopolamine, homatropine and cocaine. In these plans atropine is both synthesized and metabolized (Neumann and Tschöpe 1966). An atropine esterase for the breakdown of atropine has been demonstrated in Datura stramonium according to Kaczkowski (1964). This author also found evidence for the presence of an enzyme for the synthesis of atropine from tropic acid and tropine. Using column chromatography, it was possible to separate the esterase and the synthetase; therefore two different enzymes are involved in the synthesis and breakdown of atropine.

Vegetable atropine esterase has been studied in vitro (Jindra, Čihak, 1963) Enzyme activity is maximal at pH 5.3 and 30^0; it is inhibited by excess substrate and non-specific for one of the optical isomers of atropine.

Breakdown of tropic acid in plants is not known (Neumann and Tschöpe, 1966). However, samples of tropa-alkaloids, obtained by extraction of plant materials contain various more-basic unsaturated fatty acids, that might have been the result of breakdown of tropic acid (Flück, 1965).

[Phenylalanine and phenylpyruvic acid are direct precursors in the synthesis of tropic acid in vivo. According to Gibson and Youngken (1967) 1,3 ^{14}C phenyl pyruvic acid (C_6H_5-*CH_2-CO-*COOH) is converted in vivo in 1,2 ^{14}C tropic acid (C_6H_5-*$CH(CH_2OH)$-*COOH. This rearrangement is intriguing because it does not involve release of CO2. The rearrangement therefore occurs through an intramolecular group transfer.]

1.3 METABOLISM OF ATROPINE IN MIRO-ORGANISMS

A number of micro-organisms has been described to be able to metabolize atropine. Kedzia et al (1961) investigated the expiration date of belladonna eyedrops. He found micro-organisms in many droppers that could breakdown atropine. Such micro-organisms were found even in air samples from the hospital in Danzig and in soil samples in the surroundings. In total 53 strains were isolated, 38 of them appeared to be Pseudomonas.

The microbial decomposition of atropine has been studied by Kackowski (1959) in a bacterium isolated from soil underneath the Datura stramonium plant. The bacterium is probably an Athrobacter as mentioned by the author. In the extract of the bacterium, grown with atropine as carbon source, it was possible to demonstrate the presence of an atropine esterase: the products of hydrolysis tropic acid and tropine could be identified. Further data suggested the conversion of tropic acid in atropic acid (2-phenylacrylic acid) and the demethylation of tropine.

Niemer and Bucherer (1959, 1961) isolated a Corynebacterium named "belladonnae", able to utilize atropine as source of carbon. These authors demonstrated in this bacterium the presence of an atropine esterase and a dehydrogenase involved in the conversion of tropine into the corresponding ketone. A small amount of phenylacetic acid could be isolated from the growth medium that might have been formed by decarboxylation and dehydrogenation of tropic acid.

Also Jindra and Čihak (1963) communicated to have isolated from soil a Coryne bacterium "belladonnae" able to use atropine for its needs for carbon and nitrogen. Further details could not be found.

Published literature on the breakdown of atropine seems to be limited to the description of the hydrolysis into tropic acid and tropine; the atropine esterase involved has been demonstrated in a range of organisms. Literature data provide hardly any insight in the metabolism of tropic acid. No data are available regarding enzymes, involved in this metabolism.