The Enzymatic Hydrolysis of the β-Methyl Derivatives of Acetylcholine and Acetylthiocholine

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Since the observation made in 1938 by Glick [1] that horse serum splits only the D-isomer of acetyl-β-methylcholine, the only report on this subject has recently been published by Hoskin and Trick [2]. The latter authors, using rat brain homogenate, showed that the brain acetylcholinesterase also exhibits stereospecificity, the D-isomer only of the substrate used by Glick being split. Similar observations have been made in this laboratory with other enzyme preparations and other substrates. The results obtained will be briefly reported in the following.

The enzyme activity was measured with the Warburg technique and, in addition to acetyl-β-methylcholine, a series of thiocholine esters (as iodides) were used as substrates. Purified enzyme preparations of the electric organ of *Torpedo marmorata* and human blood plasma were employed; in addition, the esterase activity of cobra venom was similarly tested. The initial substrate concentration was 6.12 μmoles in a total reaction mixture of 2.40 ml. After complete hydrolysis of the substrate, the same amount of substrate was added to the reaction mixture from a second side bulb of the flask, and the hydrolysis reaction followed up to renewed completeness.

The results obtained with the electric tissue acetylcholinesterase (I) and human plasma cholinesterase (II) are recorded in Fig. 1. The experiments carried out with the cobra venom enzyme gave results which were identical with those obtained with the electric tissue enzyme. The enzymatic hydrolysis of a racemic mixture of acetyl-β-methylcholine (MeCh) came to completion when exactly 50 % of the amount of added substrate (6.12 μmoles) was hydrolysed. When a second portion of enzyme was added to this reaction mixture, no further hydrolysis was observed. If the same amount of substrate (MeCh) as the initial one was added to the reaction mixture after complete hydrolysis of the first added substrate (60 min for the electric tissue enzyme, 90 min for the plasma enzyme in the experiments shown in Fig. 1), hydrolysis started again, and was complete when 50 % of the second portion of substrate was hydrolysed. For comparison, the 100 % hydrolysis of acetylcholine is inserted in the figure. Human plasma cholinesterase splits acetyl-β-methylcholine at a very low rate, and therefore a 100 times higher concentration of this enzyme was used compared with the concentration used for the hydrolysis of acetylcholine. Furthermore, it was observed that after 50 % enzymatic hydrolysis of the β-methyl derivative by the plasma enzyme, the hydrolysis reaction continued with a very low rate, approximately 20 times lower than the initial rate. This infers that the plasma enzyme does split both the stereoisomers of acetyl-β-methylcholine, the hydrolysis rate for one of these (presumably the D-isomer) being about 20 times higher than that for the other isomer.

The experiments were repeated with some thiocholine esters. In all cases where thiocholine was the reaction product, the total amount of CO_2 produced was higher than expected from a theoretical point of view, when one mole of substrate hydrolysed is equivalent to one mole of CO_2 evolved from the bicarbonate system. For the thiocholine esters, CO_2 was not produced in equivalent amounts to the substrate hydrolysed, and this was probably due to the acidic property of thiocholine at the pH of the reaction mixture (7.4). Studies made by Hansen [3] with the tertiary amino analogue of thiocholine have shown that one of the two pK_a values observed for this derivative is approximately 8 which possibly may be due to the protolysis of the mercapto group. The mercapto group of thiocholine may be suggested to have similar acidic property. This, partly at least, explains the observation first made by Koelle [4], with a technique similar to that used in the present investigation, that acetylthiocholine is hydrolysed at a higher rate than acetylcholine by certain cholinesterase preparations. It may also explain the higher hydrolysis rates for the thiocholine esters at pH 8.0 than at pH 6.4, which difference is more marked than that for the corresponding reaction rates for choline esters.

Acetyl-β-methylthiocholine (MeThCh) gave similar results, 7.5 μmoles of CO_2 being produced instead of 6.12 μmoles ex-

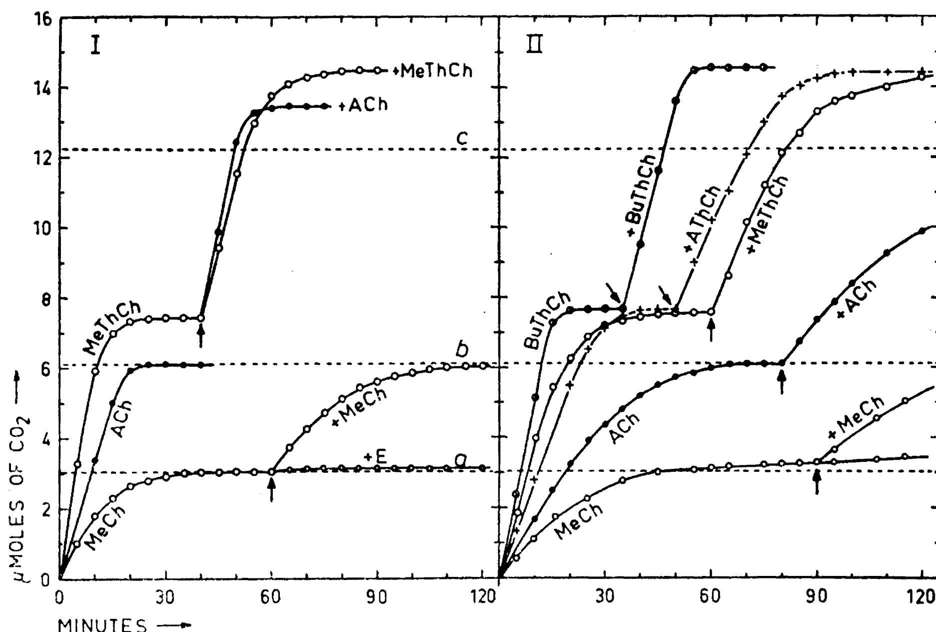

Fig. 1. The enzymatic hydrolysis of choline and thiocholine esters by purified cholinesterase preparations from *Torpedo* electric tissue (I) and human blood plasma (II). Initial amount of substrate 6.12 μmoles; total reaction mixture 2.40 ml. Arrows designate the addition of a second portion (6.12 μmoles) of substrate (or enzyme). The same enzyme concentration was used for various substrates, except in II where this concentration was 5 times higher for MeThCh and 100 times higher for MeCh. a = 3.06 μmoles; b = 6.12 μmoles; c = 12.24 μmoles. Symbols: ACh = acetylcholine chloride, MeCh = acetyl-β-methylcholine iodide, AThCh = acetylthiocholine iodide, BuThCh = butyrylthiocholine iodide, MeThCh = acetyl-β-methylthiocholine iodide. Symbols omitted for the initial hydrolysis curves in II for AThCh and MeThCh.

pected from a mole to mole basis. This optically active substrate used as racemic mixture thus behaved fundamentally different from its oxygen analogue. It has not been possible to find an explanation for this difference. It is very unlikely that the esterases show stereospecificity only for the choline esters and not for the thiocholine esters. It is more likely that the thioester is racemised during the enzymatic hydrolysis. A further difference between the S- and O-analogues of the β-methyl esters is that the former does not show the selective substrate specificity against acetylcholinesterases [5].

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