The Enzymic Hydrolysis of Triacetin by Acetylcholine-Esterase and its Inhibition by Various Compounds

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In the course of investigations on the mechanism of action of the inhibition of acetylcholine-esterase (human erythrocytes) by various compounds, triacetin has been used as a substrate. There are proofs for the existence of two active groups, centre I and centre II, of the acetylcholine-esterase molecule. Centre I is a negatively charged group which attracts the positive nitrogen atom of the choline ester. Centre II combines with the acyl group of the ester, acetates being split at a higher rate than other esters. Any acetates, acetylcholine as well as the acetates of a great variety of alcohols [1], are split. Centre II alone is responsible for this hydrolysis [2].

Acetylcholine protects acetylcholine-esterase against the action of tetraethylpyrophosphate (TEPP) [3-5]. Physostigmine also has this protective action against TEPP when acetylcholine is used as substrate. It has now been demonstrated that choline has a similar action.

Choline inhibits the enzymic hydrolysis of triacetin. As expected this inhibition is not competitive. At constant triacetin concentration the degree of inhibition increases with increasing choline concentration to about 70 per cent (corresponding to 0.25 *M* choline). Then the inhibition is constant in the presence of still higher choline concentrations. This inhibition may be due to steric hindrance.

It has been demonstrated recently that, in a mixture of acetylcholine, prostigmine or physostigmine, and acetylcholine-esterase, an equilibrium rate is reached in 10 to 25 minutes, depending upon the inhibitor concentration, after adding the inhibitor or substrate respectively. The inhibition is stronger than during equilibrium when the enzyme has been incubated with the inhibitor, it is less strong when substrate and inhibitor are added simultaneously. This is the result obtained with acetylcholine [3,4,5]. With triacetin the reaction curves are different from those obtained with acetylcholine. Equilibrium is attained immediately after adding triacetin to the incubated enzyme as well as in the case of simultaneous addition of substrate and inhibitor to the enzyme. Therefore, comparing the degrees of inhibition of the hydrolysis of acetylcholine and triacetin this fact must be considered.

Another important result has also been obtained with prostigmine and triacetin. If the concentration of this inhibitor is raised above 10^{-8} *M* keeping the substrate concentration constant, the degree of inhibition is no more increased; on the contrary, it decreases. Even with 10^{-6} *M* prostigmine the inhibition is less than with 10^{-8} *M*.

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nish tall oil does not contain linolenic acid.

This investigation will be published more in detail.

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Free Amino Acids in Brewing Materials

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The free amino acids occurring in barley, malt, wort, beer and bottom yeast *(Saccharomyces Carlsbergensis)* have been determined by means of paper partition chromatography [1]. Finely ground, six row barley (Stella) was extracted with an 8 per cent solution of trichloroacetic acid and the same extraction fluid was used on malt and yeast. The trichloroacetic acid extracts were washed with ether and purified by precipitation with 80 per cent ethyl alcohol. After filtration, the filtrate was concentrated on a boiling water bath and the concentrate used for the chromatographic separation. Wort and beer were treated with alcohol and filtered in the same way as the trichloracetic acid extracts. The beer was fermented a second time after the evaporation of alcohol and the addition of dextrose. The amino acids found are given in Table 1.

Table 1.

	Barley	Malt	Unboiled wort	Boiled wort	Beer	Beer 2nd ferm.	Yeast
Alanine	+	+	+	+	+	+	+
γ-Amino-butyric acid	+	+	+	+	+	+	+
Arginine	+	+	+	+	+	−	+
Asparagine	+	+	+	+	−	−	+
Aspartic acid	+	+	+	+	−	−	+
Cystine	+	+	?	?	?	?	+
Glutamic acid	+	+	+	+	+	+	+
Glutamine	+	+	+	+	+	+	+
Glycine	+	+	+	+	+	+	+
Histidine	+	+	+	+	+	−	+
Isoleucine	+	+	+	+	+	−	+
Leucine	+	+	+	+	+	−	+
Lysine	+	+	+	+	+	−	+
Phenylalanine	+	+	+	+	+	−	+
Proline	+	+	+	+	+	+	+
Serine	+	+	+	+	+	−	+
Threonine	+	+	+	+	−	−	+
Tyrosine	+	+	+	+	+	−	+
Valine	+	+	+	+	+	+	+
Unknown (peptides)	−	1	3	2	−	−	9

The occurrence of γ-amino-butyric acid, which has not earlier been found in brewing

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The protective action of acetylcholine, physostigmine, and choline against TEPP action (acetylcholine as substrate) lets us assume that TEPP reacts with at least one of the two active centres of the enzyme molecule. It has now been demonstrated that triacetin also protects the enzyme against TEPP action. Triacetin is attracted to centre II and, consequently, the inactivation of the enzyme by TEPP is a destruction of that centre. Furthermore, it was demonstrated that choline, reacting only with centre I, does not protect the enzyme when it is actively splitting triacetin. These results support the recent proposal [6] that the alkyl phosphates combine with the "ester-grouping" of the enzyme.

A full report will be published elsewhere in due course.

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