CHAIN LENGTH SPECIFICITY FOR PECTIN-METHYLESTERASE INHIBITION BY ANIONIC DETERGENTS[1,2]

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The possibility that plant auxins affect the methyl esterification of cell wall pectic substances has been suggested by Bennet-Clark (1955) and others. Recently Ordin, Cleland and Bonner (1957) have presented evidence that indolacetic acid (IAA) affects the rate of incorporation of methionine methyl groups into cell wall pectic substances. According to these workers, the findings support the hypothesis that esterification of carboxyl groups of pectin is involved in the auxin mechanism of cell expansion. These reports have suggested to us that a study of higher plant pectic enzymes and of chemical substances affecting their activity might provide new tools and information useful in studying possible auxin-pectic enzyme relationships.

McColloch and Kertesz (1947) reported that soaps and alkyl aryl sulfonate detergents powerfully inhibited tomato pectin-methylesterase (PM) activity *in vitro*. However, since detergents are protein-denaturing agents, it was stated that the observed inhibition might be due to this effect.

This communication reports a study of the effects of chain lengths of fatty acid salts and alkyl sulfates on their inhibition of PM.

EXPERIMENTAL

The tomato PM preparation employed was prepared by salt extraction, freeze-concentration and dialysis precipitation, essentially as described by

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McColloch, Moyer and Kertesz (1946). The final PM preparation consisted of the dialysis precipitate redissolved in 5% NaCl, and cleared of undissolved material by centrifugation. This preparation contained 59 pectin-methylesterase units (PMU) per milliliter, (McColloch and Kertesz, 1947).

Potassium salts of the fatty acids[3] were prepared in ethanolic solutions and precipitated with acetone. The potassium salts were then crystallized from ethanol-acetone solutions. Alkyl sulfates were prepared by treating the corresponding alcohols[3] with concentrated sulfuric acid. The sulfating reaction mixture was made basic with KOH and the potassium alkyl sulfates extracted with hot ethanol and allowed to crystallize. Both the fatty acid salts and potassium alkyl sulfates were washed with acetone and vacuum dried. Aqueous solutions of these compounds were prepared for subsequent use.

PM activities in the presence and absence of inhibitors were determined by the method of McColloch and Kertesz (1947), using a Beckman Model K automatic titrator. All of the determinations were conducted at pH 7.0 in 50 ml of 0.5% Pectinum NF-VII solution which was 0.1M with respect to NaCl. Inhibitor was added before adjusting the pH to 7.0, and 0.2 ml (11.8 PMU) of the enzyme preparation was used in each determination. Percent inhibition was calculated as $100 - \left(\frac{\text{PMU with inhibitor}}{\text{PMU without inhibitor}} \times 100\right)$.

RESULTS AND DISCUSSION

Table I lists the percent of PM inhibition by very small amounts of the detergents. In the case of both fatty acid salts and alkyl sulfates maximum inhibition occurs at the C_{14} chain length. Inhibition is only moderate for C_{12} compounds and practically negligible, at a concentration of 10^{-4} moles, for all other chain lengths. This chain length specificity would indicate that the inhibition of PM is not due to denaturation of the enzyme protein by the detergents.

It can also be seen in Table I that the alkyl sulfates are much stronger inhibitors of PM than are the fatty acid salts. Since the sulfates are also

[3] Fatty acids and alcohols were obtained from commercial sources.

TABLE I

Chain Length Specificity for PM Inhibition
By Fatty Acid and Alkyl Sulfate
Potassium Salts

Carbon Chain Length	Alkyl Group	Percent PM Inhibition at pH 7.0		
		Fatty Acid Salts	Alkyl Sulfates	
		1×10^{-4} M	1×10^{-4} M	5×10^{-5} M
10	Capryl	5	0	--
12	Lauryl	11	100	40
14	Myristyl	57	100	100
16	Palmityl	7	21	--
18	Stearyl	0	0	--

stronger acids it seems that a strongly anionic group as well as a specific chain length is required for maximum inhibition.

Our results suggest that the ability of fatty acids to inhibit PM depends upon the chain length and configuration of such acids. It is tempting to speculate that plants may be able to regulate PM activity *in situ* by metabolic control of the amount and proportion of certain naturally occurring fatty acids.

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