Lysozymes' esterase activity

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A non-specific esterase activity of hen lysozyme (c-type) has previously been described: histidine was claimed to be involved in this activity. The present letter reports that a histidine-less lysozyme from the duck egg-white (c-type) as well as a goose lysozyme (g-type) possess such an esterase activity determined using *p*-nitrophenylacetate.

Lysozyme *Esterase activity* *Duck lysozyme* *Goose lysozyme*

1. INTRODUCTION

Lysozymes (EC 3.2.1.17) are defined as β-1,4-*N*-acetylmuramidases cleaving the glycosidic bond between the C-1 of *N*-acetylmuramic acid and the C-4 of *N*-acetylglucosamine in the bacterial peptidoglycan. Lysozymes are ubiquitous enzymes [1]; the most intensively investigated are those from avian egg-white and there are two distinct types of enzyme from this source: hen egg-white lysozyme (chicken-type, c-type) and goose egg-white lysozyme (g-type) [1–3]. The existence of other distinct types of lysozyme (from plants, invertebrates, phages) has been demonstrated: they differ from the c- and g-types on the basis of structural, catalytic and immunological criteria [1]. The c-type lysozymes have been characterized at high concentration only in two orders of birds, the Galliformes and the Anseriformes [4] but they have also been detected in mammals [5], reptiles [6] and insects [7]. The most studied c-type lysozyme is hen egg-white lysozyme and Phillips' group [8] established that its catalytically active functional groups are Glu (no.35) and Asp (no.52). Some studies have been devoted to the determination of a possible catalytic function of lysozyme other than its lytic action: Piszkiewicz and Bruice [9] found that hen lysozyme catalyzes the hydrolyses of 6 carboxylic esters in an apparently bimolecular reaction. The esterase activity toward esters such as *p*-nitrophenylacetate was described to be dependent on the presence of histidine (residue no.15): indeed carboxymethylation of this residue was claimed to lead to the loss of the esterase activity. This observation was corroborated in [10].

In the course of our studies devoted to various c-type enzymes, we encountered lysozymes which were devoid of histidine [11]. This note reports that not only these histidine-less lysozymes but also g-type lysozymes present an esterase activity.

2. MATERIALS AND METHODS

Hen egg-white lysozyme was from Boehringer. Duck lysozyme II and ostrich lysozyme were prepared according to [12,13]. *p*-Nitrophenylacetate was purchased from Sigma and all other reagents were from Merck or Prolabo.

Esterase activity was determined at 26°C using *p*-nitrophenylacetate as in [14] except that 50 mM Tris–HCl (pH 7.5 and 8) and 50 mM sodium acetate (pH 5) buffers were used. Ester concentration was 2×10^{-4} M while enzyme concentration

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Table 1

p-Nitrophenol formation (%) during the action of 3 lysozymes at pH 5, 7.5 and 8 and 26°C on *p*-nitrophenylacetate (2×10^{-4} M)

Lysozyme		pH 5			pH 7.5			pH 8		
Origin	Conc. (M)	20 min	60 min	120 min	20 min	60 min	120 min	20 min	60 min	120 min
Hen	1.65×10^{-5}	trace	trace	trace	<1	2	2	2	5	9
	3.3×10^{-5}				2	3	3	3	7	10
	1.0×10^{-4}				5	11	14	9	20	26
Duck II	1.65×10^{-5}	trace	trace	trace	4	5	7	6	9	12
	3.3×10^{-5}				7	9	9	11	15	20
	1.0×10^{-4}				12	21	31	32	46	54
Ostrich	3.3×10^{-5}	n.d.	n.d.	n.d.	8	11	14	n.d.	n.d.	n.d.

Control experiments have been performed in the absence of the enzymes; trace amounts, <1%; n.d., not determined

varied (0.165×10^{-4} up to 10^{-4} M): they were similar to values in [9]. *p*-Nitrophenol formation was observed at 348 nm after different time intervals. Control experiments were carried out in the absence of the enzymes.

3. RESULTS AND DISCUSSION

We confirm in table 1 that hen lysozyme has a weak esterase activity at pH 7.5 and 8 (but not at pH 5): indeed it was necessary to employ larger enzyme quantities to demonstrate this property. Surprisingly, we observed a slightly more pronounced esterase activity with duck lysozyme II [12] which is devoid of histidine. Table 1 shows also that not only c-type but also g-type lysozymes share this behaviour as ostrich lysozyme [13,15] possesses also a slight esterase activity (at pH 7.5).

Work in [9,10] suggested that the unique histidine residue (no.15) of hen lysozyme was implicated in its esterase site as carboxymethylation of this amino acid led to loss of esterase activity. Duck lysozyme II is quite homologous to hen lysozyme [12]: however, it possesses no histidine and a leucine residue in position 15. Thus the loss of hen lysozyme's esterase activity after carboxymethylation might not be due to a modification of the unique histidine residue but probably to other modifications which may have been overlooked.

Ostrich lysozyme has only a weak and quite localized sequence homology with hen lysozyme [15]. However, the three-dimensional structures of g- and c-type lysozymes present striking similarities [16]: there are 90 spatially equivalent α-carbons in goose and hen lysozymes; to the amino-terminal helix (residues no. 4–16) of hen lysozyme which contains the unique histidine residue coincide residues no. 48–60 of goose lysozyme devoid of this amino acid [16]. In conclusion, further studies seem necessary to delineate the amino acid(s) implicated in lysozymes' weak and perhaps non-specific esterase activity.

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