SUBUNIT WEIGHT AND *N*-TERMINAL GROUPS OF LIVER AND KIDNEY CARBOXYLESTERASES (EC 3.1.1.1)

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1. Introduction

During the past decade considerable progress has been made in the isolation of highly purified carboxylesterases and in the evaluation of their molecular properties (for a review see [1]). The molecular weight of pig liver esterase, as independently determined by ultracentrifugation in three laboratories, is 163,000–168,000 [2–4]. The equivalent weight of unspecific carboxylesterases was estimated by titration with organophosphorus inhibitors, and found to be in the order of 80,000. It is assumed, therefore, that these esterases contain two active sites [3, 5–7] This is consistent with the reported subunit composition, since dissociation of pig and ox liver esterase into active half molecules of a molecular weight of 75,000–85,000 has been found in several laboratories [3, 4, 6].

Whether there is a further dissociation into quarter molecules, e.g. polypeptide chains of a molecular weight of ∿40,000 in the presence of concentrated urea, guanidine-HCl, and SDS [4, 9], is still uncertain [3]. In addition, nothing is known yet about the possible identity or non-identity of the subunits.

In this paper the molecular weight of subunits from three carboxylesterases from different origin is reported as determined by disk electrophoresis in SDS, gel filtration in the presence of 6 M guanidine-HCl, and quantitative analysis of the *N*-terminal groups.

Abbreviations:
FDNB: 1-fluoro-2,4-dinitrobenzene;
DNP- : dinitrophenyl-;
SDS : sodium dodecylsulfate.

2. Methods

Carboxylesterases from pig liver, pig kidney, and ox liver were isolated as described previously [6, 7]. For the determination of the subunit weights, the enzyme preparations were subjected to disk electrophoresis in SDS as reported by Weber and Osborne [10] and Dunker and Rueckert [11]. The problem of band overlap between markers and the esterases was circumvented by using the split gel technique [11, 12].

Quantitative determination of *N*-terminal groups: the carboxylesterases were dinitrophenylated in the presence of 8 M urea, precipitated with HCl and washed with acetone and ether. After addition of the ^{14}C-labelled DNP-amino acids as internal standards [13], the preparation was hydrolysed 5 or 16 hr at 105° in 6 N HCl. For the qualitative detection of the *N*-terminal groups, other appropriate conditions of hydrolysis were employed. The ether-soluble fractions of the DNP-amino acids were separated by thin-layer chromatography using the solvent systems of Brenner et al. [14]. The amount of DNP-glycine and DNP-leucine was determined at 366 nm after elution with 15% ethanol. The corresponding molar extinction coefficients were found to be 16,880 ($M^{-1}cm^{-1}$) for DNP-glycine (m.p. 206°) and 17,630 for DNP-leucine (m.p. 132°). The actual concentration of protein in the hydrolysate of a known amount of DNP-enzymes was determined by quantitative analysis of alanine [13, 15]; the theoretical amount was then calculated from the amino acid content of the esterases [2]. ^{14}C was measured in a Packard liquid scintillation counter with a dioxane scintillation mixture.

Gel filtration was carried out on Sephadex G 150

using a column 2.5 X 50 cm. The enzyme samples (volume 0.5 ml) were applied to the column immediately after dilution to the corresponding concentrations. For elution 0.05 M tris-HCl containing 0.1 M KCl + 0.02% NaN_3 was used. In each run dextran blue and DNP-alanine were applied together with the enzyme and their corresponding elution volumes used for the calculation of the K_d-values [16].

For the gel filtration experiments in 6 M guanidine [17] see the legend to fig. 2.

3. Results

3.1. *Disk electrophoresis on polyacrylamide gel in the presence of SDS*

The subunit weights of carboxylesterases from pig liver, pig kidney, and ox liver were determined by disk electrophoresis in SDS [10, 11]. Using 8 known proteins for calibration, a linear relationship between electrophoretic mobility and the decadic logarithm of the molecular weight was obtained. All three carboxylesterases showed one main band migrating between the H-chain of γ-globulin (M.W. 50,000) and bovine serum albumin. This is illustrated by fig. 1. The quantitative evaluation of the results gave values of about 63,000 for all three enzymes (table 1). In addition, one weak and slower migrating band was detected with an estimated molecular weight of 117,000 ± 7,000 (ox liver esterase; 14 determinations) and 121,600 ± 7,600 (pig liver esterase; 5 determinations), respectively. This corresponds approximately to the double of the subunit weight reported above. In several cases a very weak third band with a mobility corresponding to the trimer was observed.

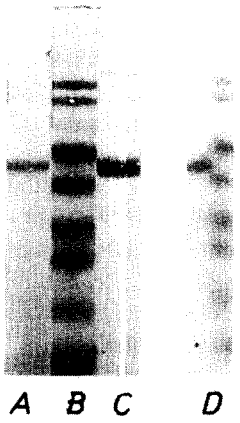

Fig. 1. SDS-disk electrophoresis on polyacrylamide gel. A) pig liver esterase; B) marker proteins, from top to bottom: β-galactosidase, phosphorylase A, ovalbumin dimer (weak), bovine serum albumin, γ-globulin (H-chain), ovalbumin, pepsin, γ-globulin (L-chain), β-lactoglobulin; C) ox liver esterase; D) comparative run of the same marker proteins and of ox liver esterase, split gel technique [12].

3.2. *Gel filtration in 6 M guanidine*

In additional experiments the three esterases were carboxymethylated in the presence of 6 M guanidine and subjected to column chromatography on agarose-gel in 6 M guanidine according to Fish et al. [16]. The column was calibrated with 8 known proteins of a molecular weight range from 12,400 to 138,000 which were carboxymethylated in the presence of 6 M guanidine before analysis (fig. 2). All three carbox-

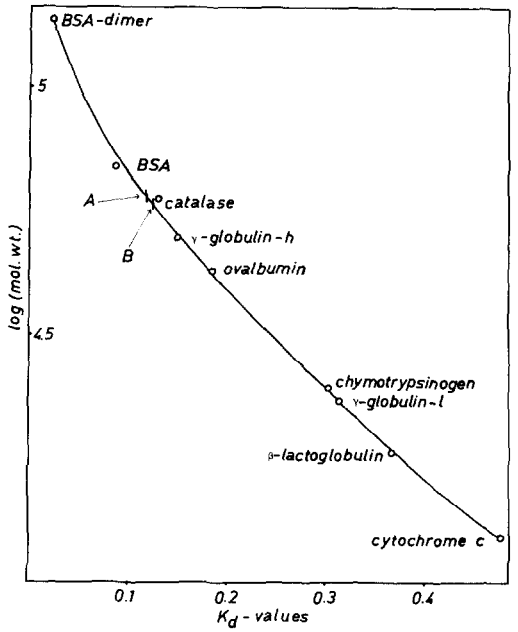

Fig. 2. Estimation of chain weight by gel filtration on biogel A 5 M in 6 M guanidine-HCl [17]. K_d-values [16, 17] from a typical series of 12 runs (20.0 °C); column 90 X 1.9 cm; each point represents the means of at least 2 K_d-values. The proteins were carboxymethylated in 6 M guanidine [17] before the run; A = ox liver esterase; B = pig liver esterase, BSA = bovine serum albumin.

Table 1
Subunit weight of carboxylesterases.

Method	Carboxylesterase from		
	Pig liver	Ox liver	Pig kidney
Disk electrophoresis in SDS	63,470 ± 1670 (7)	63,120 ± 1750 (22)	62,700 ± 900 (6)
Gel filtration (Biogel) in 6 M guanidine	61,000 ± 2,800 (4)	61,400 ± 1,300 (4)	58,500 ± 6,000 (4)
Quantitative determination of *N*-terminal groups	Gly 61,700 63,300 (2)	Leu 58,600 52,000 (2)	Gly 61,200 52,100 (2)

The values represent the average ± standard deviation; number of experiments in brackets.

ylesterases behaved almost identically as monomeric catalase. The values found for different esterase preparations are shown in table 1. There was no evidence for a second peptide chain with a different molecular weight.

No difference in the elution volume was found between beef liver esterase, which had been neither carboxymethylated nor treated with mercaptoethanol, and the carboxymethylated enzyme derivative. This indicates, that disulfide bonds are not involved in the association of the subunits.

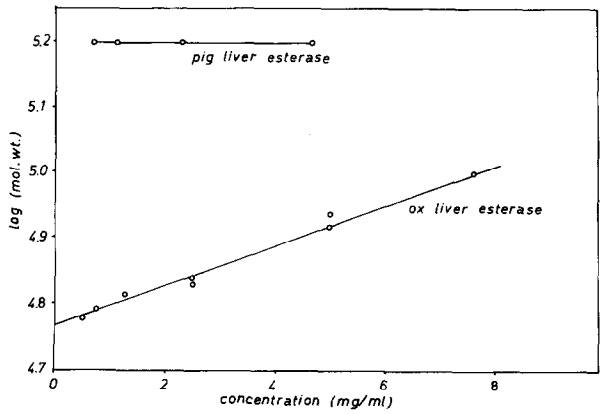

Fig. 3. Gel filtration of pig and ox liver esterase on Sephadex G 150. Dependence of the apparent molecular weight on the concentration of enzyme protein (semilogarithmic plot).

3.3. *Quantitative analysis of the N-terminal groups with FDNB*

After condensation of the carboxylesterases from pig liver and kidney with FDNB only DNP-glycine was found in the hydrolysate (5 hr, 105°, 6 N HCl), whereas in the carboxylesterase from beef liver leucine was the only *N*-terminal group (hydrolysis: 16 hr, 105°, 6 N HCl). For a quantitative determination of these groups, ^{14}C-labelled DNP-leucine or DNP-glycine was added before hydrolysis of the dinitrophenylated protein as an internal standard [12]. From the recovery of radioactivity after the chromatographic purification of the DNP-derivatives, factors were calculated, which allowed a correction of the losses occurring during the hydrolysis and purification procedure. In all experiments one mole of end-group per about 60,000 *g* of protein was found. These results are also listed in table 1.

3.4. *Gel filtration on Sephadex G 150*

The elution volume of native ox liver esterase, when subjected to gel filtration in the absence of SDS or urea, shows a marked dependency on the protein concentration. This has already been observed, though not studied systematically, by Runnegar et al. [8]. In fig. 3 the apparent molecular weights of ox liver esterase, as obtained by comparison of the K_d-values with those of known proteins, are plotted against the enzyme concentration. By extrapolation of the curve

to a protein concentration of zero, a minimal molecular weight of 55,000–60,000 is obtained. This is in satisfactory agreement to the chain weights reported above (table 1). In contrast, pig liver esterase, in a concentration range of 0.7–4.6 mg/ml, gives a constant K_d-value (fig. 3), corresponding to a molecular weight of about 160,000, which is in reasonable agreement with previous ultracentrifugation data [2–4]. This confirms earlier observations that ox liver esterase dissociates far more readily, e.g. at higher protein concentrations, than the pig liver enzyme [6].

4. Discussion

The results presented in this communication give evidence that the chain weight of the carboxylesterases studied is about 60,000. The agreement between the four different methods used was reasonable. There was no indication for any other larger or smaller species, for instance of a molecular weight ∿40,000 or ∿80,000. The three carboxylesterase preparations of different origin behaved identically. Our results are inconsistent with the present assumption of a dimer, composed of two active half-molecules of a molecular weight about 80,000 [3, 4, 6]. It now seems more probable, that the carboxylesterases from pig liver, pig kidney, and ox liver (M.W. 162,000–168,000) are composed of three subunits of identical chain weight. There still remains some discrepancy, however, between the molecular weight as determined by ultracentrifugation [2–4] and the three-fold subunit weight (∿180,000). Ecobichon has recently reported that all esterase isozymes of a crude extract of bovine liver have the same molecular weight of 53,000–55,000, as determined by gel filtration. He assumes, therefore, that in the course of the purification process the enzyme associates to a trimeric form [18]. – It is difficult, however, to reconcile the assumption of three subunits with the reported number of two active sites. A possible explanation could be either that one of the three subunits, while having the same molecular weight and the same *N*-terminal group as the others, does not bear an active site, or that the equivalent weights obtained by titration with organophosphate or carbamate inhibitors may, for some unknown reason, be considerably too high. Further experimental work will be required to clarify this point. –

In the determination of the *N*-terminal groups only one major DNP-amino acid has been found in all three enzymes, indicating that the subunits are identical in this respect. Pig liver and kidney esterases have the same *N*-terminal group, glycine, while in ox liver esterase leucine has been found. This is in agreement with previous findings, which have revealed a close relationship between pig liver and pig kidney esterase, whereas the enzyme from ox liver differs in the amino acid composition and "fingerprint" peptide maps [2] as well as in the amino acid sequence of an active site peptide [19].

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