HIGH-MOLECULAR-WEIGHT DEOXYRIBONUCLEASE FROM *VERONGIA AEROPHOBA*

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1. Introduction

Most of the known DNA-hydrolyzing enzymes (EC 3.1.4.5. – 3.1.4.9., Deoxyribonucleases, DNases) have been isolated from bacteria and from mammalian tissues [1]. In order to find DNases with enzymatic properties different to the known species we are investigating DNases from lower marine organisms in particular from *Verongia aerophoba* (Schmidt), a marine sponge. Marine organisms have been chosen as starting material (1) because of their abundant availability, (2) on account of the considerable enzyme activity of the crude extract, and (3) because enzymes from marine organisms may be expected to have some unusual properties.

To our knowledge up to now in marine organisms there is only one report on a DNase from *M. mercenaria*, a marine clam [2] and one on several DNases from the hepatopancreas of *Octopus vulgaris* [3].

During the purification of DNase from *V. aerophoba* the molecular weight of the enzyme seemed to be considerably higher than 17.000–38.000 as reported for DNases so far [4–6]. This prompted us to a molecular weight determination the results of which are presented in this paper.

2. Materials and methods

The sponges were harvested and dried at Rovinj, Yugoslavia. Enzyme extraction and purification by ammonium sulphate fractionation, gel-chromytography on Sephadex G-200 and isoelectric column electrophoresis (electrofocusing) will be published elsewhere. The purified enzyme used in these experiments had a specific activity of 300 Kunitz units/mg protein.

Aldolase from rabbit muscle, catalase from bovine liver, cytochrome *c* from horse heart, bovine serum albumin and egg-albumin as molecular weight standards, and the auxiliary enzymes for the aldolase assay were purchased from Boehringer-Mannheim, 8132 Tutzing, Germ. Molecular weight determinations by density gradient centrifugation were performed in sucrose gradients essentially according to Martin and Ames [7]. Centrifugations were run with a MSE Superspeed 65 preparative ultracentrifuge using a 3 X 21 ml swing-out rotor with 10 ml adapters at 30.000 rpm (G_{max} 129.000 *g*) and 4° C. The gradients were checked by refractometry in each fraction with the Abbé-refractometer (Fa. Zeiss, 7082 Oberkochen, Germ.). Since the enzyme is unstable in alkaline solution and centrifugation even at ph 7–8 resulted in major losses and inconsistent sedimentation profiles, the pH of the sucrose gradients (5–20%, 0,1 M sodium acetate) was brought to 5.0 which is near the pH-optimum of the *Verongia*-DNase.

DNase activity was measured spectrometrically as published by Kunitz [8]. Catalase was assayed by following the decrease in absorbancy of H_2O_2 at 240 nm [9], aldolase was measured by the coupled enzyme assay using α-glycerophosphate dehydrogenase and triosephosphate isomerase according to Bücher et al. [10]. Bovine serum albumin and egg

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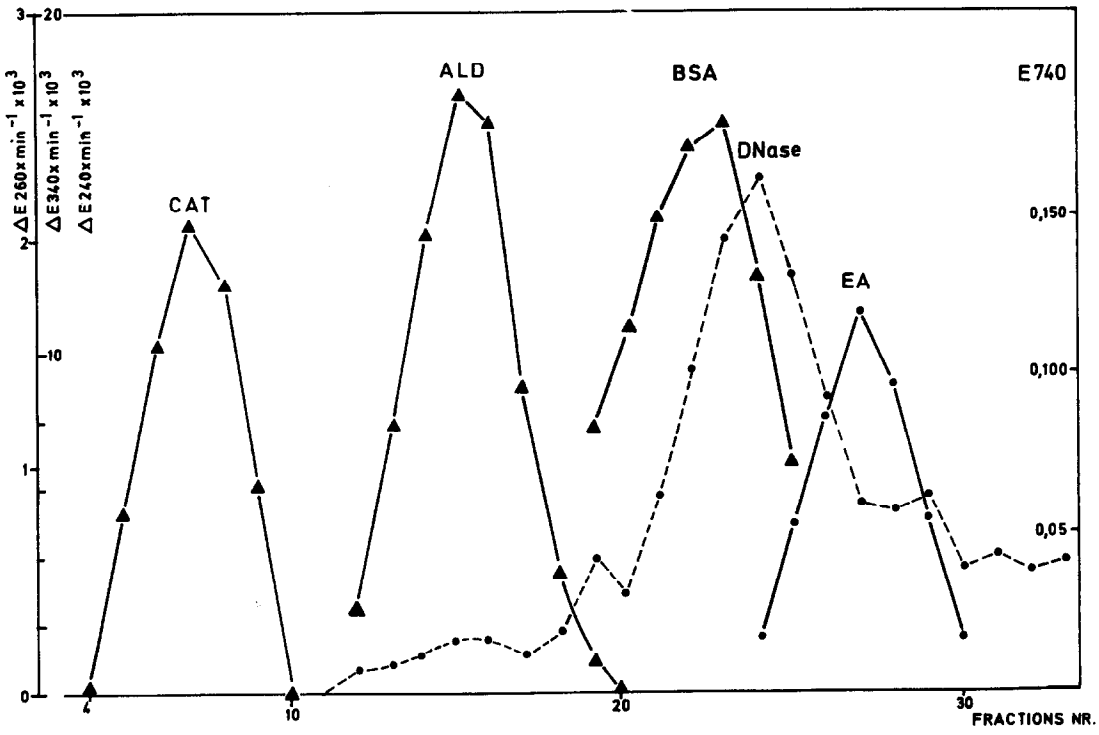

Fig. 1. Sucrose gradient sedimentation profile of catalase (CAT, 100 μg), aldolase (ALD, 100 μg), bovine serum albumin (BSA, 100 μg), egg-albumin (EA, 100 μg/ml) and DNase from *Verongia aerophoba* (DNase, 80 μg protein). Centrifugation at 30.000 rpm (G_{max} 129.000 g) and 4° C for 21 hr. Linear sucrose gradient 5–20%, 0,10 M sodium acetate, pH 5,0), gradient volume 8,0 ml, sample layer 0.20 ml (1,3 mm), fraction volume 0.24 ml. Determination of enzyme activity, protein concentration and density were made with 10–100 μl aliquots. The top of the gradient is on the right.

Table 1
Molecular weight of DNase from *Verongia aerophoba* by sucrose density gradient centrifugation.

Reference molecular weight	Molecular weight calcualted for DNase
Aldolase (147.000)	65.500
Egg-albumin (45.000)	62.700
Bovine serum albumin (67.000)	57.500
Average ± s	61.900 ± 4.100

albumin were located in the gradient effluents by micro-precipitation with the appropriate antiserum (provided by Behringwerke, 355 Marburg, Germ.) and protein measurements according to Lowry [11].

3. Results

Preliminary runs confirmed that the rate of centrifugation for DNase as well as for the reference proteins used was very nearly constant from 5 to 35 hr, optimal resolution being obtained within 21 to 29 hr at 30.000 rpm. As can be seen in fig. 1 *Verongia*-DNase has a sedimentation constant close to that of bovine serum albumin (mol wt 67.000). Assuming the same partial specific volume for DNase and standard proteins respectively an average molecular weight of 62.000 can be calculated. The values

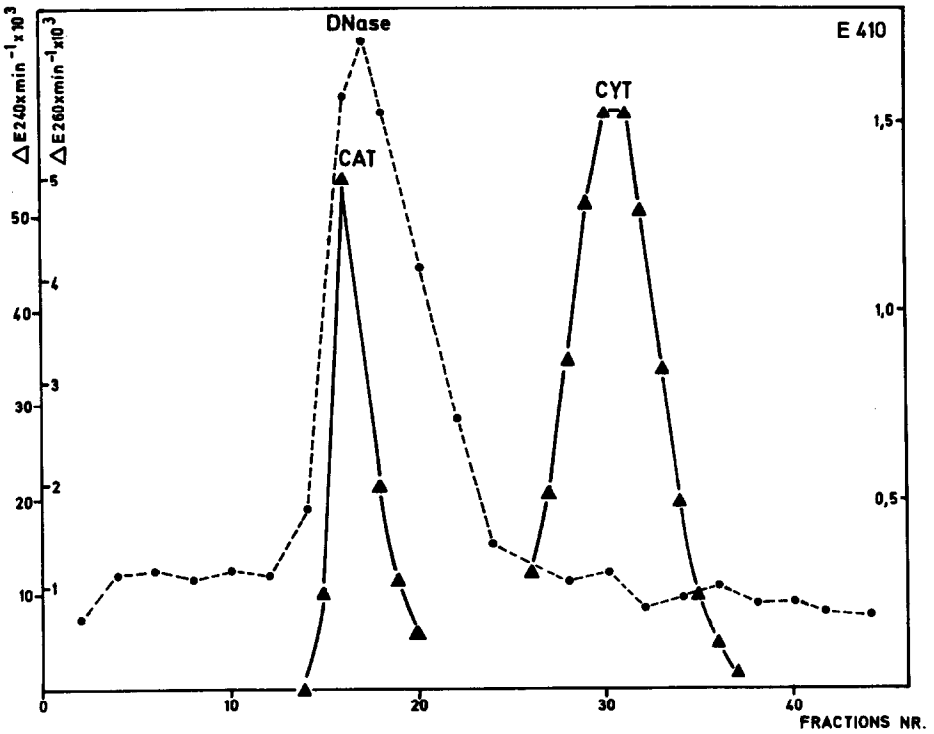

Fig. 2. Chromatography on Sephadex G-75 of DNase from *Verongia aerophoba*. Column dimensions were 1,1 X 80 cm, V_0 = 29.6 ml (determined with catalase), fraction volume 1.85 ml. The column was loaded with approx. 1 mg of catalse (CAT), cytochrome *c* (CYT) and DNase respectively in several subsequent runs, solutions were buffered with 0.1 M sodium acetate pH 5.0, in case of catalase (IEP ~5.0) pH 7.0.

obtained with different standards are listed in table 1.

From chromatography on Sephadex G-75 (fig. 2) a molecular weight of 65.000 could be derived using the equation: $\log M = 5.624 - 0.864\,(V_e/V_0)$ where V_e is the solute effluent volume and V_0 is the void volume [12]. This is in rather good agreement with the value of 62.000 obtained with gradient centrifugation.

Thus the DNase from *Verongia aerophoba* seems to have a considerably higher molecular weight than the other DNases reported so far (*Staphylococcus aureus* 17.000) [5], bovine pancreas 31.000 [6 and 1 in contrast to 8], hog spleen 38.000 [4].

Further characterization of the enzyme – especially concerning enzyme-inhibitor interaction – is in progress and will be published later.

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