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# Molecular weight of a purified acid phosphatase allozyme (ACPH[4]) from *D. virilis*.

**Narise, Sumiko, and Hiroko T. Kitagawa.** Laboratory of Biochemistry, Department of Chemistry, Faculty of Science, Josai University, Sakado, Saitama, 350-0295 Japan.

Acid phosphatases (ACPH, EC3.1.3.2) have been found in every organism studied to date. In

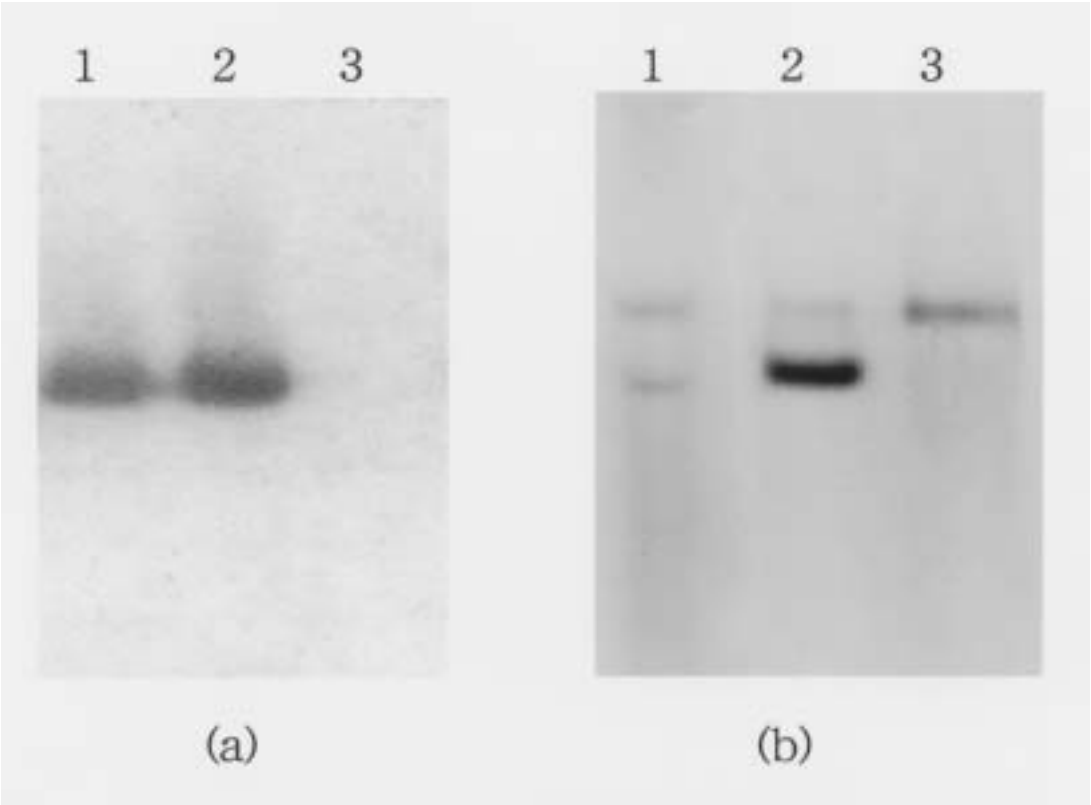


Figure 1. Native-slub PAGE of ACPH[4] stained for enzyme activity (a) and protein (b). Electrophoresis was done at pH 4.3 in a 7.5% polyacrylamide gel. Lane 1, partially purified ACPH[4] from CM column. Lanes 2 and 3, protein peaks with and without ACPH activity from HPLC.

*Drosophila*, ACPH has been studied from genetical and biochemical points of view (MacIntyre, 1966, 1971; Feigen *et al.*, 1980; Narise, 1984; Narise and Tominaga, 1987). Biochemical studies have indicated that ACPH in *Drosophila* is a homodimer with a subunit molecular weight of 55,000 by MacIntyre (1971) and of 50,000 by Feigen *et al*. (1980) for *D. melanogaster*, and of 50,000 by Narise (1984) for *D. virilis*. In these experiments the estimation of molecular weight was carried out using a partially purified enzyme and by means of gel-filtration and SDS-electrophoresis. Therefore, it can be said that these values may contain a considerable error. Recently, the nucleotide sequence of Acph-1 cDNA from *D. melanogaster* was determined (Chung *et al.*, 1996), and a molecular weight of the ACPH calculated from the sequence was 50,189.

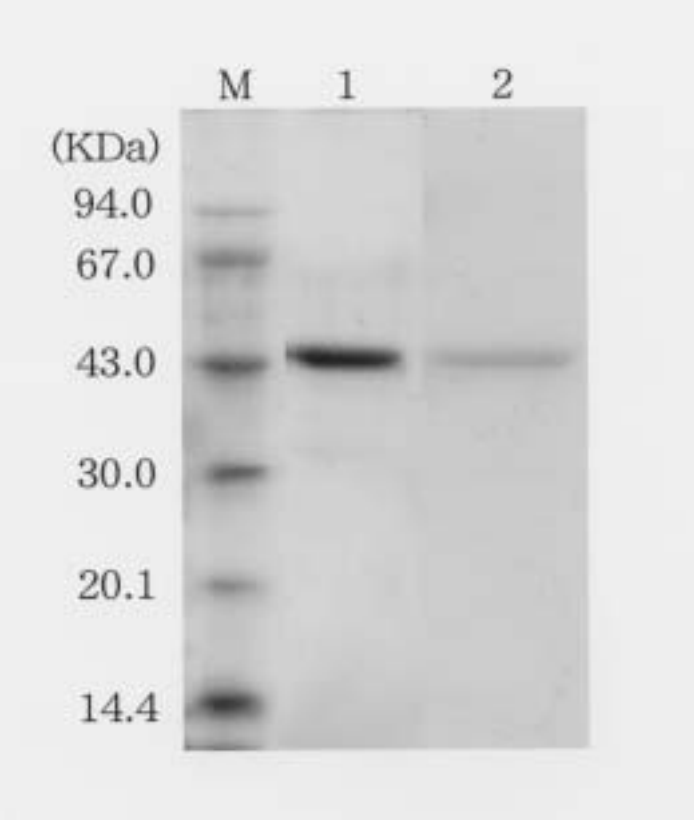


Figure 2. SDS-PAGE of the purified ACPH[4]. Lane M, The molecular weight markers (Amersham Pharmacia Biotech., Inc.). Numerals indicate the molecular weight (KDa) of phosphorylase b, bovine serum albumin, ovalbumin, carbonic anhydrase, trypsin inhibitor, and α-lactoalbumin from top. Lanes 1 and 2, purified ACPH[4] stained for protein and for glycoprotein (Pro-Q™, Fuchsia Glycoprotein Gel Stain kit, Molecular Probes, Inc.), respectively.

In regard to human acid phosphatases, nascent lysosomal enzymes have a signal peptide which functions in transportation of the enzyme protein across the endoplasmic reticulum membrane and is afterward cleaved. Furthermore, the lysosomal acid phosphatase has an additional C-terminal sequence which contains the transmembrane domain, and is lacking in the secretory prostatic acid phosphatase (Peters et al., 1989). The *D. melanogaster* Acph-1 is a glycoprotein and seems to be a lysosomal enzyme, as compared with other several mammalian acid phosphatases (Feigen et al., 1980). In *D. virilis*, four electromorphs specified by alleles (Acph[1], Acph[2], Acph[3] and Acph[4]) at Acph locus have been detected (Ohba, 1977). Previous study showed that the ACPH allozymes are also glycoproteins containing mainly neutral sugars and are anchored to lysosomes, and the ability of the allozymes to be incorporated into lysosomes is varied (Narise, 1985; Narise and Tominaga, 1987). ACPH[4] allozyme is very easily released from the lysosomes (Narise, 1985). Considering these facts, it will be necessary to estimate an exact molecular weight of these enzyme proteins. This report deals with enzyme purification of ACPH[4] and the determination of its exact molecular weight.

Enzyme source is ACPH[4] electromorph bearing Acph[4] allele in *D. virilis*. 240g of adult flies were homogenized and the supernatant after centrifugation was referred as crude extract. After successive protamine and acid treatment, the following column chromatographies were sequentially used; hydroxyapatite, Sephadex G-100, and CM-52. The sample from the CM-52 chromatography, though showing more than 300 fold purification of the crude extract, exhibited two bands on the native PAGE, one of which had ACPH activity, as shown in Figure 1, lane 1. Therefore, the ACPH[4] protein was separated by use of a cation exchange HPLC (POROS HS column, PerSeptive Biosystems). It is quite evident from lanes 2 and 3 in Figure 1 that the impurities have been removed from ACPH[4] by the HPLC. 240g flies yielded 1.6 mg ACPH[4] protein of approximately 1000 fold purification with a recovery of about 6%. Estimation of the molecular weight of the purified ACPH[4] was carried out by SDS-PAGE and MALDI-TOF mass spectrometry. Figure 2 shows an example of SDS-PAGE of ACPH[4]. Several experiments demonstrated that ACPH[4] has a 43,000-47,000 Dalton of molecular weight and is a glycoprotein (Figure 2). Figure 3 shows MALDI-TOF mass spectra of the purified ACPH[4]. ACPH[4] exhibited major protein peaks (Mr 43911.9) for a

subunit (monomer) and (Mr 88214.5) for a dimer. Taking the limit of measurement error (0.06%) into account, the molecular weight of the ACPH[4] seems to be about 44,000 Dalton for a subunit.

Nevertheless, this estimated molecular weight is about 6,000 Dalton smaller than that of the primary structure deduced from the nucleotide sequence of Acph-1 gene (Chung et al., 1996). Chung et al. (1996), in comparison with the human lysosomal ACPH, estimated the first 33 residues to be a N-terminal signal peptide and the last 36 residues, a C-terminal, respectively. If it is true, the 44,000 molecular weight of ACPH[4] seems to be reasonable, since the ACPH[4] released from lysosomes (mature ACPH) might have no signal peptides.

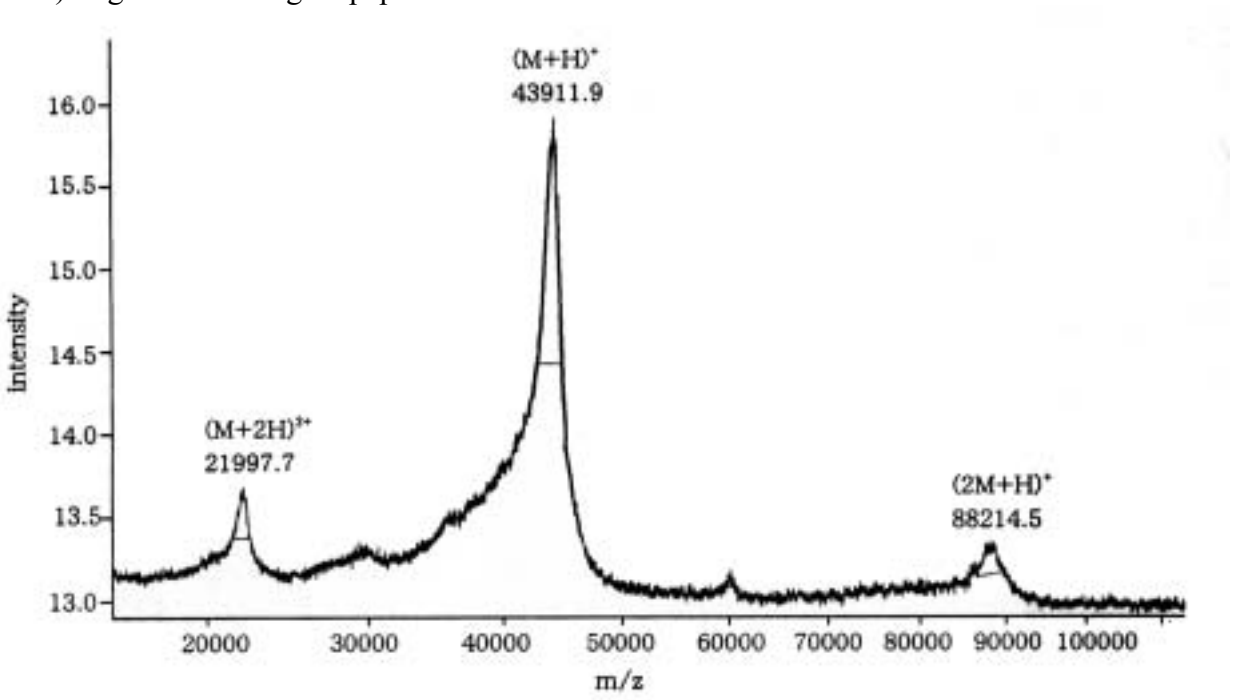


Figure 3. MALDI-TOF mass spectra of the purified ACPH[4]. MALDI-TOF mass was carried out using a Finnigan MAT, VISION 200 system. The instrument was externally calibrated using the $[M+H]^+$ ion peak of aldolase (MW 39153.1) and DHBs matrix [2,5-Dihydroxybenzoic acid: 5-methoxysalicylic acid (9:1)].

Reference: Chung, H-J., C. Shaffer, and R. MacIntyre 1996, Mol. Gen. Genet. 250: 635-646; Feigen, M.I., M.A. Johns, J.H. Postlethwait, and R.R. Sederoff 1980, J. Biol. Chem. 255: 10338-10343; MacIntyre, R.J., 1966, Genetics 53: 461-474; MacIntyre, R.J., 1971, Genetics 68: 483-508; Narise, S., 1984, Insect Biochem. 14: 473-480; Narise, S., 1985, Genet. Res. Camb. 45: 143-153; Narise, S., and H. Tominaga 1987, Biochem. Genetics 25: 415-428; Ohba, S., 1977, Population Genetics. UP Biology Series, Tokyo University Press, Tokyo, pp. 99-104; Peters, C., C. Geier, R. Pohlmann, A. Waheed, K. von Figura, K. Poiko, P. Virkkunen, P. Henttu, and P. Vihko 1989, Biol. Chem. Hoppe-Seyler 370: 177-181.