Crystallisation and preliminary X-ray analysis of the 'common-type' acylphosphatase

Marjolein M.G.M. Thunnissen[a], Elisha G. Agango[a], Niccolò Taddei[b], Gianfranco Liguri[b], Cristina Cecchi[b], Alessandro Pieri[b], Giampietro Ramponi[b], Pär Nordlund[a,*]

[a]*Department of Molecular Biology, University of Stockholm, Stockholm, Sweden*
[b]*Department of Biochemical Sciences, University of Florence, Florence, Italy*

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Abstract Single crystals of a 'common-type' acylphosphatase from bovine testis have been grown. Crystals belong to space group C2 and have cell dimensions $a = 64.6$ Å, $b = 36.5$ Å, $c = 45.2$ Å and $\beta = 104.8$ and contain one monomer per asymmetric unit. The crystals diffract better than 2.0 Å resolution and are well suited for an X-ray structure determination.

Key words: Acylphosphatase; Protein crystallisation; X-ray diffraction; Structure determination

1. Introduction

Acylphosphatase (EC 3.6.1.7) is a very basic cytosolic enzyme generally constiting 98 amino acid residues (M_r about 11,000), recently extensively reviewed [1]. It catalyses the hydrolysis of acylphosphates of both synthetic and physiological interest such as carbamoyl phosphate, 1,3-bisphosphoglycerate, and β-aspartylphosphate of phosphorylated proteins [2]. Data is accumulating to support a role for the enzyme in the regulation of cation transport across membranes [3] as well as in the regulation of the glycolytic pathway [4]. Moreover, a possible role for acylphosphatase in inositol lipid metabolism in platelets has been suggested [5]. Acylphosphatase has been found in many organs from different vertebrate species as two isoenzymatic forms called the 'common-type' (also known as 'erythrocyte') and 'muscle' forms, respectively, depending on their tissue distribution. Although the two isoenzymes feature similar kinetic properties and substrate specificity, they show only 55% sequence homology [6,7].

Previous attempts at crystallising the muscle isoenzyme only yielded small needle-shaped crystals which were not suitable for X-ray studies. The structure of the horse muscle acylphosphatase has instead been determined in solution by ^{1}H nuclear magnetic resonance (^{1}H NMR) spectroscopy [8,9]. The enzyme is an α/β protein composed of a five-stranded antiparallel β-sheet and two parallel α-helices. The secondary structural elements can be tentatively grouped in two $\beta\alpha\beta$ subdomains intercalated into each other, giving a 4-1-3-2 β-strand topology. A similar folding topology has been found in other proteins such as the activation domain of procarboxypeptidase B [10], the RNA binding domain of the nuclear ribonucleoprotein A [11], and the histidine-containing phosphocarrier protein [12]. All the above mentioned proteins are involved in phosphate binding and it has been suggested that they could contain a common phosphate binding motif [13]. Despite such folding similarity, neither a significant sequence homology nor functional relationship have been shown between these proteins.

Although the NMR study of the muscle acylphosphatase revealed the three-dimensional fold of the acylphosphatase family, still very little is known about which residues constitute the active site and therefore the structural basis for its catalytic action.

In this paper we report crystals suitable for high-resolution X-ray analysis and a preliminary structural characterisation of the 'common-type' acylphosphatase isolated from bovine testis [14].

2. Materials and methods

The 'common-type' acylphosphatases was purified as previously described [14]. The chemicals used for the crystallisation experiments were purchased from Merck and were of the highest purity available. Crystallisation conditions for the protein were initially searched by using the sparse matrix approach [15], in hanging drop vapour diffusion set-ups in cell culture plates at 4°C. Two conditions giving visible crystals were subsequently reproduced and optimised using a finer grid search. X-ray diffraction data were collected on a MAR-research imaging plate system mounted on a Siemens rotating anode.

3. Results and discussion

Crystals of the 'common-type' acylphosphatases grow in a broad range of pHs and PEGs. The best crystals were grown in a 0.25 M actetate buffer, pH 3.5, 0.2 M ammonium sulphate and either 30% PEG 8000 or 30% PEG 4000 using a protein concentration of 4 mg/ml. This condition yields plate-like shaped crystals (Fig. 1), approximately $0.1 \times 0.3 \times 0.3$ mm within 1 week. The crystal parameters are described in Table 1. The volume of the unit cell corresponds well with the assumption that there is one acylphosphatase molecule per asymmetric unit. The V_m value is then 2.4 Å^3/Da which is in the normal range for protein crystals [16]. The crystals diffract to better than 2.0 Å when using an in-house rotating anode X-ray source and a graphite monochromator and are relatively stable in the X-ray beam. A native acylphosphatase data set, extending to 2.15 Å resolution, has been collected from one crystal using a Siemens Area detector system mounted on a Rigaku rotating anode. The data collected from one crystal was processed with the program XDS [17]. The resulting data set has 24,325 observations of which 5267 are unique. The data have an R-merge of 6.35% and a completeness of 95% (79.4% complete for data between 2.2 and 2.15 Å resolution).

We conclude that the crystals of the 'common-type' acylphosphatase, presented in this paper, are well suited for high

*Corresponding author.

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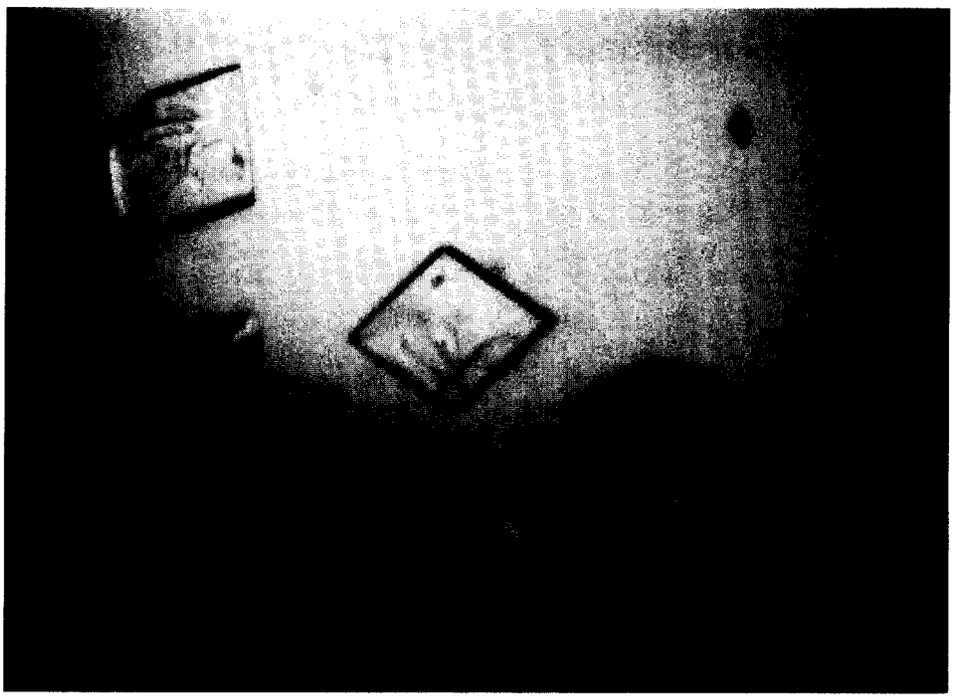

Fig. 1. Crystals of 'common-type' acylphosphatase in a hanging drop.

Table 1
Cell parameters of the acylphosphatase crystals

Space group	Cell (Å)	β angle	Diffraction limit (Å)
C2	64.6 36.5 45.2	104.8	2.15

resolution crystallographic studies which should be helpful in the determination of the reaction mechanism for acylphosphatases.

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