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CARBOHYDRATE COMPOSITION AND PHYSICAL PROPERTIES OF TOBACCO PHOSPHODIESTERASE

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Abstract—Some physical and chemical properties of phosphodiesterase from cultured tobacco cells were studied. The enzyme contained *ca* 50% carbohydrate consisting of residues of arabinose, glucose, glucosamine, galactose, mannose and xylose. Analyses showed that the enzyme had a sedimentation coefficient, $s_{20,w}$, of 16 S, a Stokes' radius of 5.7 nm and a calculated specific volume of 0.66 ml/g. The MW of the enzyme was calculated to be 300 000 from these values.

INTRODUCTION

Previously we reported the purification and properties of a new phosphodiesterase isolated from cultured tobacco cells [1, 2]. The enzyme also shows pyrophosphatase activity, but it does not hydrolyse DNA or RNA. The enzyme preferentially cleaves the pyrophosphate bond of the 5′-terminal methylated blocked structure in eukaryotic *m*RNA without splitting a main chain in the RNA molecule. Thus the enzyme has been used for studies on the identification and biological function of the blocked terminal structure in a nucleic acid molecule [3–5].

The results of the previous investigations indicated that the enzyme is a tetrameric glycoprotein [2, 6]. The purposes of the present study were to characterize its chemical and physical properties further.

RESULTS AND DISCUSSION

The results of amino acid and carbohydrate analyses indicate that essentially all of the sample weight (98%) is accounted for by amino acid and carbohydrate residues, of which 48% is represented by amino acid residues and 50% by carbohydrate. The results show that the neutral sugar content of the enzyme is much more than that previously reported [2]. The previous value was estimated by the orcinol–sulfuric acid method using mannose as a standard.

Table 1. Carbohydrate composition of tobacco phosphodiesterase

Carbohydrate	Residues g/100 g	Residues Number/g subunit MW (MW = 72000)
Arabinose	27.2	148
Xylose	0.5	3
Mannose	1.1	5
Galactose	3.1	14
Glucose	13.3	59
N-Acetylglucosamine	4.5	16

Analytical determination of individual sugar residues is shown in Table 1. Arabinose and glucose are the major neutral sugar residues. The hexosamine in the enzyme was identified as glucosamine by GC; no galactosamine was found.

Arabinose and glucose are not usually found in oligosaccharides linked via an *N*-glycosidic bond of *N*-acetylglucosamine to asparagine in the polypeptides [7].

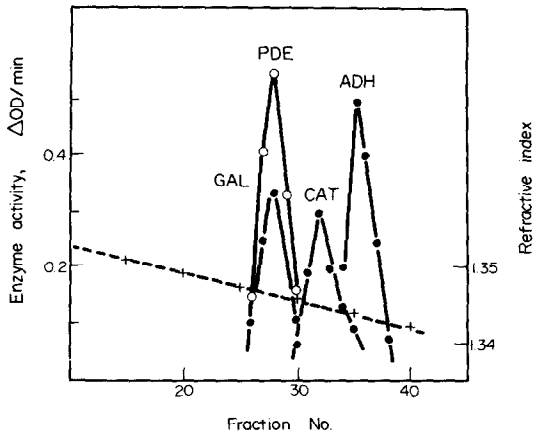

Fig. 1. Sedimentation of tobacco phosphodiesterase in a sucrose gradient. PDE, tobacco phosphodiesterase; GAL, *E. coli* β-galactosidase; CAT, beef liver catalase; ADH, yeast alcohol dehydrogenase.

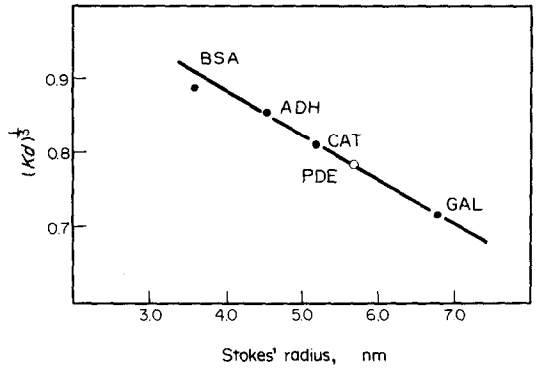

Fig. 2. Stokes' radius of tobacco phosphodiesterase as determined by gel filtration on Sepharose 6B. BSA, bovine serum albumin; ADH, yeast alcohol dehydrogenase; CAT, beef liver catalase; PDE, tobacco phosphodiesterase; GAL, *E. coli* β-galactosidase.

Arabinose residues in plant glycoproteins are commonly linked to hydroxyproline [8], whereas the tobacco phosphodiesterase contains no hydroxyproline [2]. Whether these sugar residues are present in side chains attached by *N*-acetylglucosamine or are linked directly to the polypeptide via an *O*-glycosidic bond to amino acid hydroxyls remains unknown and awaits further analysis.

The enzyme was sedimented through 5–20% sucrose gradient in the presence of marker enzymes with known $s_{20,w}$ values (Fig. 1). The enzyme co-sedimented with β-galactosidase, and the sedimentation coefficient ($s_{20,w}$) of the enzyme was estimated to be 16 S.

The Stokes' radius of the enzyme was determined by gel filtration according to Porath [9]. As shown in Fig. 2, the Stokes' radius of the enzyme was estimated to be 5.7 nm.

From amino acid and carbohydrate analyses a partial specific volume of 0.66 ml/g was calculated [10, 11]. Using the sedimentation coefficient, Stokes' radius and partial specific volume, the MW of the enzyme was calculated to be 300000 [12], which is in good agreement with the previously reported value determined by gel filtration [1]. The frictional ratio was calculated to be 1.33 using the sedimentation coefficient, Stokes' radius and partial specific volume [12]. The physical properties of the enzyme are summarized in Table 2.

Previous results indicated that the enzyme was a tetrameric glycoprotein having an identical subunit, and that it was possible to prepare, through incubation of the enzyme with urea and 2-mercaptoethanol, the monomer of the enzyme which was stable and catalytically active [6]. Usually dissociated subunits of oligomeric enzymes reassociated into oligomeric structures under non-denaturing conditions, but dissociated subunits of the tobacco phosphodiesterase did not [6]. The present results show that the enzyme has an unexpectedly high carbohydrate content. It may be possible that carbohydrates attached to polypeptide chains prevent reassociation of the enzyme subunits into oligomers. The quaternary structure of the enzyme may be formed before the polypeptide chains are fully glycosylated.

Table 2. Properties of tobacco phosphodiesterase

Parameter	Value	Method	Ref.
Molecular weight	280000	Gel filtration	1
	300000	Calculated from $s_{20,w}$, Stokes' radius and $\bar{V}$	
Subunit molecular weight	72000	Sodium dodecyl sulfate gel electrophoresis	6
	74000	Gel filtration	6
Subunit number	4	Cross-linking experiment	6
Sedimentation coefficient, $s_{20,w}$	16 S	Sucrose gradient centrifugation	
Stokes' radius	5.7 nm	Gel filtration	
Partial specific volume	0.66 ml/g	Chemical composition	
Frictional ratio	1.33	Calculated from $s_{20,w}$, Stokes' radius and $\bar{V}$	
Isoelectric point	8.8		2

EXPERIMENTAL

Enzyme preparation. Phosphodiesterase was isolated from cultured tobacco cells and purified to a homogeneous state on gel electrophoresis as described in ref. [1].

Chemical analysis. Amino acids were analysed using an amino acid analyser as described in ref. [2]. For determination of neutral sugar, the enzyme was hydrolysed in 2 N TFA at 100° for 2 hr. The hydrolysate was dried under red. pres. and passed through a column of Dowex 50 (H^+) coupled to a column of Dowex 1 (formate). Neutral sugars in the eluate were reduced and acetylated as described in ref. [13]. The acetylated alditol derivatives were analysed by GC using a packed column described in ref. [14] with *myo*-inositol as int. standard. Hexosamines were determined following hydrolysis of the enzyme in 4 M HCl at 100° for 6 hr. After removal of HCl, the hydrolysate was passed through a column of Dowex 50 (H^+) and hexosamines were eluted with 2 N HCl. The alditol acetates were prepared and analysed by GC using a glass column packed with 3% Poly A-103 on Gas Chrom Q [15]. The hexosamine content was estimated by the Elson–Morgan method [16] using glucosamine–HCl as a standard and the values reported as *N*-acetylglucosamine.

Sucrose gradient centrifugation. Sucrose gradient, 5–20% in 10 mM Tris–HCl (pH 7.5) containing 0.2 M NaCl, was centrifuged in a SW-41 rotor at 39000 rpm for 6 hr. Samples containing tobacco phosphodiesterase, yeast alcohol dehydrogenase ($s_{20,w}$ = 7.4 S), beef liver catalase (11.4 S), and *E. coli* β-galactosidase (16.0 S) were layered on top of the gradient. Marker enzymes were assayed as described in refs. [17–19].

Gel filtration. A column (1.6 × 70 cm) of Sepharose 6B was equilibrated and eluted with 10 mM Tris–HCl buffer (pH 7.5) containing 0.2 M NaCl. The marker proteins used and their Stokes' radii are BSA (3.6 nm), alcohol dehydrogenase (4.6 nm), catalase (5.2 nm) and β-galactosidase (6.8 nm).

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