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Crystallization and preliminary X-ray diffraction studies of the *Saccharomyces cerevisiae* phospholipid-transfer protein Sec14p

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Abstract

The *Saccharomyces cerevisiae* phosphatidylinositol-transfer protein Sec14p catalyzes the exchange of phosphatidylinositol or phosphatidylcholine between membrane bilayers *in vitro*, and is an essential protein required for the budding of secretory vesicles from the yeast Golgi complex *in vivo*. At issue is the fundamental question of how the dual phospholipid ligand specificity of Sec14p translates to *in vivo* function. In an attempt to determine the structural basis for how Sec14p binds each of its phopholipid ligands, Sec14p occupied with phosphatidylcholine has been purified and the complex crystallized in the presence of the mild detergent *n*-octyl β-D-glucopyranoside. The Sec14p crystals diffract to 2.7 Å and belong to space group $P3_121$ or $P3_221$ with unit-cell dimensions of $a = b = 88.79$, $c = 111.21$ Å, $\alpha = \beta = 90$, $\gamma = 120°$. As Sec14p exhibits significant primary sequence homology to mammalian retinaldehyde binding proteins and the noncatalytic domain of human MEG2 protein tyrosine phosphatase, is is anticipated that solution of the Sec14p crystal structure will provide new functional insights for a family of interesting proteins.

1. Introduction

The *Saccharomyces cerevisiae* Sec14p (304 amino-acid residues, $M_r = 35$ kDa) belongs to a ubiquitous family of proteins, the phosphatidylinositol transfer proteins, that facilitate the energy-indpendent transport of phosphatidylinositol (PI) and phosphatidylcholine (PC) between membrane bilayers *in vitro* (Bankaitis, Aitken, Cleves & Dowhan, 1990). *In vivo*, Sec14p function is essential for transport of secretory glycoproteins from a late Golgi compartment and for cell viability (Bankaitis, Aitken, Cleves & Dowhan, 1990; Cleves, McGee & Bankaitis, 1991; Bankaitis, Malehorn, Emr & Greene, 1989; Cleves, McGee, Whitters, Champion, Aitken, Dowhan, Goebl & Bankaitis, 1991). Specifically, Sec14p appears to be required for the biogenesis of Golgi-derived secretory vesicles and, consistent with such a function, Sec14p localizes as a peripheral Golgi membrane protein (Bankaitis, Malehorn, Emr & Greene, 1989). Recent studies of the mechanisms by which Sec14p functions to stimulate Golgi secretory function indicate that Sec14p senses the PC content of yeast Golgi membranes and that the PC-bound form of Sec14p effects a negative feedback regulation on CDP-choline pathway activity (McGee, Skinner, Whitters, Henry & Bankaitis, 1994; Skinnner, McGee, McMaster, Fry, Bell & Bankaitis, 1995). The proposed purpose of this regulation is to spare a Golgi diacylglycerol (DAG) pool from nonproductive, and ultimately lethal, consumption *via* PC biosynthesis (Alb, Melissa & Bankaitis, 1996; Kearns, McGee, Mayinger, Gedvilaite, Phillips, Kagiwada & Bankaitis, 1997). Other data suggest the possibility that the PI-bound form of Sec14p serves to stimulate Golgi PI turnover; thereby providing a potential mechanism for Sec14p to also contribute to Golgi DAG production (Kearns, McGee, Mayinger, Gedvilaite, Phillips, Kagiwada & Bankaitis, 1997). Thus, the two phospholipid-bound forms of Sec14p are proposed to execute complemetary, yet independent, actions to ensure the existence of sufficient Golgi DAG to sustain secretory vesicle biogenesis *via* the Sec14p pathway. Precisely how DAG functions to stimulate secretory vesicle assembly remains unknown, but it likely plays a regulatory role that somehow acts through Kes1p, a yeast member of the human oxysterol binding protein family that negatively regulates progression of signal through the Sec14p pathway (Fang, Kearns, Gedvilaite, Kagiwada, Kearns, Fung & Bankaitis, 1996).

While the sensor model suggests specific functions for the PI- and PC-bound forms of Sec14p in preserving Golgi DAG, it does not provide any insight into the quantitative contribution of each liganded form of Sec14p to Golgi function. Experimental investigation of this issue requires the generation of mutant Sec14ps that are specifically defective in either PI or PC binding, yet various mutagenesis strategies have failed to generate such interesting classes of mutants. For this reason, we have undertaken a crystallographic approach to the problem with the hope that structural analysis of specific Sec14p–phospholipid complexes will reveal the mechanisms by which Sec14p recognizes/binds the cognate phospholipid headgroups. Herein, we report the successful crystallization of the phosphatidylcholine-bound form of Sec14p and preliminary characterization of the corresponding crystals.

2. Materials, methods and results

2.1. *Overexpression and purification of Sec14p*

Hexahistidine-tagged Sec14p was expressed to high levels in *E. coli* KK2186 cells using vector pQE-31. The correct DNA sequence was determined *via* the dideoxy chain-termination method (Sanger, Nicklen & Coulson, 1977). Cells were grown to an optical density of 1.2 at 595 nm and induced with 0.5 m*M* isopropyl β-D-thiogalactoside (IPTG) for 3 h at 310 K. Sec14p overexpressing cells were collected, resuspended in ice-cold lysis buffer [50 m*M* sodium phosphate (pH 7.1), 300 n*M* sodium chloride, 10 m*M* β-mercaptoethanol, 1 m*M* NaN_3, 0.2 m*M* phenylmethylsulfonylfluoride (PMSF)] and disrupted in a bead beater (Biospec Products). The homogenate was serially clarified at 5000*g*, 12000*g* and 100000*g*. Sec14p was precipitated at 50% saturation with ammonium sulfate, precipitates were resuspended in lysis buffer, dialyzed, loaded onto a column of Ni^+-NTA resin

Table 1. *Data statistics*

Data set	Native	K_2PtCl_4	$PbAc(CH_3)_3$
Resolution (Å)	2.7	3.0	3.0
Unique reflections	14275	10230	10984
R_{symm} (%)†	6.2	6.0	5.8
R_{symm} of the outer shell (%)	26.5	25.5	22.4
Completeness of the outer shell (%)	99.4	95.8	93.3
Redundancy	3.0	2.6	3.4
R_{def} (%)‡		25.2	19.5
Number of binding sites		4	1
Phasing power		1.60	1.04
R_{Cullis}		0.71	0.82
Combined figure of merit	0.496		

† $R_{symm} = \sum_i \sum_{hkl} |I_{i,hkl} - \langle I_{hkl} \rangle| / \sum_i \sum_{hkl} \langle I_{hkl} \rangle$, where hkl is the unique reflection index, $I_{i,hkl}$ is the intensity of symmetry redundant reflections and $\langle I_{hkl} \rangle$ is the mean intensity. ‡ $R_{def} = \sum_{hkl} |F_{PH} - F_P| / \sum_{hkl} |F_P|$, where F_{PH} and F_P are structure amplitudes of heavy-atom derivatives and native crystals, respectively.

(Qiagen), and eluted with a linear gradient of imidazole (0–200 m*M*) in lysis buffer. Peak fractions were subsequently collected, and dialyzed exhaustively against lysis buffer. Sec14p was then occupied with egg phosphatidylcholine (Avanti Polar Lipids) by incubation at room temperture for 3–4 h in the presence of at least a 100:1 molar ratio of PC:Sec14p with PC presented in the form of unilamellar vesicles. Protein was rebound to a Ni^+-NTA column eluted with a linear imidazole gradient as before, peak fractions pooled, and the preparation dialyzed exhaustively against 10 m*M* HEPES (pH 7.0), 150 m*M* KCl, 10 m*M* 2-ME, 1 m*M* NaN_3, 0.2 m*M* PMSF. Finally, Sec14p was further purified by gel-filtration chromatography using a Superdex-75 column (Pharmacia) mounted onto a Pharmacia fast protein liquid crystallography system. The purified Sec14p was fully active for PI and PC transfer as determined by the appropriate phospholipid transfer assays (data not shown).

2.2. *Crystallization and preliminary crystallographic analysis*

Purified PC-bound Sec14p was concentrated to 20 mg ml^{-1} in 10 m*M* HEPES (pH 7.2), 150 m*M* KCl. Diffraction quality crystals of dimensions of 0.8 × 0.8 × 0.8 mm were grown by the hanging-drop vapor-diffusion method within 2 d at room temperature. The well solution consisted of 10 m*M* HEPES (pH 7.2), 150 m*M* KCl, 2% *n*-octyl β-D-glucopyranoside and 3% PEG 3350, and the hanging drops had a 1:1 ratio of protein to well solution. The volume of the drop is 4 µl. Consequently, the final detergent concentration in the drop was 1%. Native data were collected on a DIP 2030 image plate using 1.0° oscillation and 25 min exposure time at room temperture, 50 images were processed by the *HKL* package (Minor, 1993; Otwinowski, 1993). The crystals belong to space group $P3_121$ or $P3_221$ with unit-cell dimensions of $a = b = 88.79$, $c = 111.21$ Å, $\alpha = \beta = 90, \gamma = 120°$. The native data set is 99.2% complete at 2.7 Å resolution with a R_{symm} of 0.062. The calculated V_m is 3.6 for one monomer per asymmetric unit, corresponding to 65% solvent content in the unit cell, a value within the range normally observed for protein crystals (Matthews, 1968). The large solvent content should help to improve phase quality when solvent flattening is applied. There was no indication of oligomerizaton by self-rotation analysis (data not shown).

2.3. *Heavy-atom derivatives*

The Sec14p crystal structure will have to be solved by the multiple isomorphous replacement (MIR) method because no similar structures are available. Two useful heavy-atom derivatives were prepared by soaking Sec14p crystals for 12 h in individual well solutions that contained 2 m*M* of K_2PtCl_4 or $PbAc(CH_3)_3$, respectively (Fig. 1). The diffraction data for these heavy-atom derivatives were collected on a Siemens Histar multiwire area detector, and data were processed by the *X-GEN* program. Heavy-atom positions were located from isomorphous difference Fourier analysis as executed by the *Xtalview* 3.0 program (McRee, 1992) and further refined by the *Mlphare* program from the *CCP*4 package (Collaborative Computational Project, Number 4, 1994). Some statistics relevant to the analysis of these derivatives are given in Table 1.

2.4. *Summary*

The *Saccharomyces cerevisiae* phospholipid-transfer protein Sec14p has been purified and crystallized. The crystals diffract to 2.7 Å resolution. Determination of the atomic structure of Sec14p complexed with PC will provide the first step in the

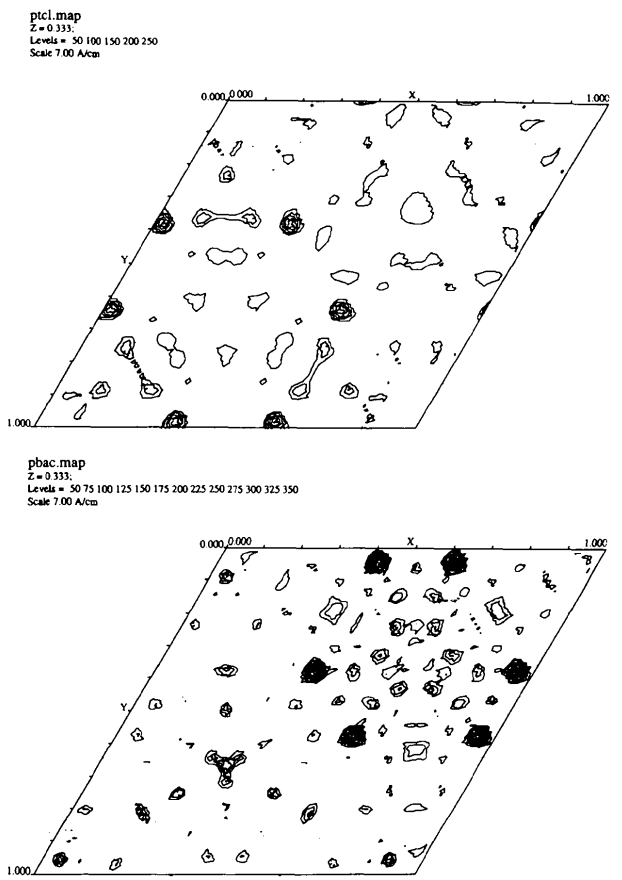

Fig. 1. Two Harker sections ($z = 0.333$) from the different Patterson maps showing clear Harker peaks for heavy-atom derivatives K_2PtCl_4 and $PbAc(CH_3)_3$. The top one is for K_2PtCl_4 and the bottom one is for $PbAc(CH_3)_3$.

solution of how Sec14p binds/recognizes its phospholipid ligands. We hope that such information will drive the directed creation of mutant Sec14ps with specific defects in PI and PC binding. Functional analysis of such mutant Sec14ps will reveal how this protein employs its phospholipid binding/exchange properties in the integration of phospholipid metabolism with secretory vesicle biogenesis, metabolic regulation of phospholipid biosynthetic pathways and organelle function in general. To our knowledge, there exists no information regarding the structure of any phospholipid transfer protein that exhibits ligand specificity. Thus, we anticipate the Sec14p crystal structure to exhibit unique and interesting features.

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