Crystal structure of carbonic anhydrase CaNce103p from the pathogenic yeast Candida albicans

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Abstract

Background: The pathogenic yeast Candida albicans can proliferate in environments with different carbon dioxide concentrations thanks to the carbonic anhydrase CaNce103p, which accelerates spontaneous conversion of carbon dioxide to bicarbonate and vice versa. Without functional CaNce103p, C. albicans cannot survive in atmospheric air. CaNce103p falls into the β-carbonic anhydrase class, along with its ortholog ScNce103p from Saccharomyces cerevisiae. The crystal structure of CaNce103p is of interest because this enzyme is a potential target for surface disinfectants.

Results: Recombinant CaNce103p was prepared in E. coli, and its crystal structure was determined at 2.2 Å resolution. CaNce103p forms a homotetramer organized as a dimer of dimers, in which the dimerization and tetramerization surfaces are perpendicular. Although the physiological role of CaNce103p is similar to that of ScNce103p from baker’s yeast, on the structural level it more closely resembles carbonic anhydrase from the saprophytic fungus Sordaria macrospora, which is also tetrameric. Dimerization is mediated by two helices in the N-terminal domain of the subunits. The N-terminus of CaNce103p is flexible, and crystals were obtained only upon truncation of the first 29 amino acids. Analysis of CaNce103p variants truncated by 29, 48 and 61 amino acids showed that residues 30–48 are essential for dimerization. Each subunit contains a zinc atom in the active site and displays features characteristic of type I β-carbonic anhydrases. Zinc is tetrahedrally coordinated by one histidine residue, two cysteine residues and a molecule of β-mercaptoethanol originating from the crystallization buffer. The active sites are accessible via substrate tunnels, which are slightly longer and narrower than those observed in other fungal carbonic anhydrases.

Conclusions: CaNce103p is a β-class homotetrameric metalloenzyme composed of two homodimers. Its structure closely resembles those of other β-type carbonic anhydrases, in particular CAS1 from Sordaria macrospora. The main differences occur in the N-terminal part and the substrate tunnel. Detailed knowledge of the CaNce103p structure and the properties of the substrate tunnel in particular will facilitate design of selective inhibitors of this enzyme.

Keywords: Carbonic anhydrase, Candida albicans, Crystal structure, CaNce103p, Substrate tunnel
nucleophilic species attacks the CO₂ molecule bound in the active site cavity [3, 4].

Based on sequence and structural features, CAs can be divided into six categories: α, β, γ, δ, ξ and η [4]. The best-studied group, the α-class CAs, were first discovered in vertebrate erythrocytes and later found in prokaryotes, protozoa and plants [4, 5]. β-CAs were first discovered in red clover and fern chloroplasts [6], and their presence has been reported in all other types of organisms except for mammals. Both α and β-CAs have been found in fungi, and all yeast CAs characterized to date belong to the β-class [3].

β-CAs are active as homodimers or higher oligomers formed by these homodimers, with one zinc atom per monomeric subunit [4, 7]. Of the four yeast CAs that have been structurally characterized, two—those from Saccharomyces cerevisiae and Cryptococcus neoformans—have a dimeric structure. Structures of two CA isoenzymes from Sordaria macrospora display tetrameric organization [8].

Candida albicans, the most common human fungal pathogen, possesses at least one gene encoding a CA [9]. This gene has been denominated NCE103 because of its homology to S. cerevisiae gene NCE103 (non-classical export). ScNCE103 was first identified as a coding sequence for a protein detected in the extracellular space, although it lacks the classical signal peptide [10]. The protein product, ScNce103p, was later found to have CA activity and is particularly important for yeast cells in environments with low CO₂ concentrations [11, 12].

Compared with non-pathogenic yeasts, C. albicans can proliferate in a more diverse range of environments. It can thrive on host skin, mucosa or blood, and it can survive on abiotic surfaces [13]. These sites differ in composition, accessibility of nutrients, pH, and concentration of gases. The CO₂ concentration in human blood is 150-fold higher than in atmospheric air, and C. albicans can adapt to both. CA is crucial for this adaptation [3].

versely, in the presence of 5.5% CO₂, NCE103 alleles is not viable in atmospheric air. Consequently, C. albicans can adapt to both. CA is crucial for this adaptation [3].

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Fig. 1 Multiple alignment of fungal β-CAs and secondary structure prediction of CaNce103p. Secondary structure prediction of CaNce103p and sequence multialignment of CaNce103p and CAs from Candida parapsilosis (CpNce103p, CGID: CAL0000147197), Saccharomyces cerevisiae (ScNce103, Swiss-Prot: P53615), Sordaria macrospora (CAS1, Swiss-Prot: C1L335), Cryptococcus neoformans (Can2, EMBL: Q314V7). The predicted secondary structure regions are indicated as follows: H-Helix, E-Sheet, T-Turn, C-Coil. Black and gray backgrounds denote identical and similar amino acid residues, respectively. Secondary structural elements (X-ray CaNce103p) found in Δ29_CaNce103p are shown as helices and arrows (strands). The black arrows indicate sites of truncation. All sequences were obtained from NCBI databases. Prediction of secondary structure and multialignment was performed using the programs CFSSP [16], MultAlin [28] and ESPript [29].
to keep the protein in a soluble state during the purification process. All CaNce103p variants were purified (Fig. 2a), with average yields of 12–23 mg purified protein per liter of culture for CaNce103p, Δ29_CaNce103p and Δ48_CaNce103p and 2–8 mg/L for Δ61_CaNce103p.

The activity of full-length and truncated versions of CaNce103p detected using the stop-flow pH/dye indicator method [15] (Fig. 3) indicated that truncation by 29 and 48 amino acids did not cause differences in the enzyme activity in comparison with the wild-type full-length CaNce103p. Truncation by 61 amino acids rendered the enzyme inactive. The reaction in presence of Δ61_CaNce103p has a similar rate as the spontaneous hydration of CO₂. The activity detection also confirmed that β-Mercaptoethanol does not negatively influence the enzyme activity.

Assessment of the oligomeric structure of Δ29_CaNce103p

Structures of fungal CAs solved to date indicate that these oligomeric enzymes are composed of an even number of identical subunits. We determined the oligomeric state of CaNce103p variants using size-exclusion chromatography. Δ29_CaNce103p formed tetramers (Fig. 3b), which was the highest-order oligomer observed in this study. Δ29_CaNce103p was the only version present as a monomer, dimer and tetramer under our experimental conditions. WT_CaNce103p occurred only as a dimer and tetramer. Δ48_CaNce103p occurred only as a monomer and a precipitate; Δ61_CaNce103p was present only as a precipitate. These findings suggest the importance of the N-terminus for folding and oligomerization of CaNce103p.

Protein crystallization

Protein crystallization was facilitated by removal of the first 29 amino acids. Our attempts to crystallize full-length CaNce103p or the variants truncated by 48 or 61 residues were unsuccessful. WT_CaNce103p, Δ48_CaNce103p and Δ61_CaNce103p were compared to the rate of spontaneous CO₂ hydration without enzyme addition.
Δ61_CaNce103p formed precipitates or very small crystals with skin on the drop. Repeated unsuccessful attempts in a variety of crystallization conditions resulted in our decision to focus on Δ29_CaNce103p. Purified Δ29_CaNce103p was enzymatically active and crystallized in the form of needles belonging to the Space Group P2₁2₁2₁ (Tab. 2), which allowed determination of the structure at 2.2 Å resolution.

Overall architecture
The overall structure of CaNce103 is similar to that of CAS1, a β-CA from the plant fungal pathogen Sordaria macrospora [8]. It also resembles structures of β-CAs from red algae [17] and bacteria including Escherichia coli, Vibrio cholerae and Haemophilus influenzae [18–20]. CaNce103 is a complex of four identical subunits organized as a dimer of dimers, in which the dimerization and tetramerization surfaces are mutually perpendicular (Fig. 4a). The subunits in each dimer are interlocked by their N-terminal arms, consisting of two perpendicular helices stretched from the rest of the molecule over the neighboring subunit (Fig. 4b, c). Each monomer provides more than 90 residues to make contact with its dimerization partner, creating an interface of 3458 Å². We calculated the interaction energy stabilizing the dimer to be −48.8 kcal/mol. Association of two dimers in a tetramer is not as strong; it relies on 33 residues forming an interface of 996 Å². The tetramer is stabilized by an interaction energy of −11.2 kcal/mol.

The central part of each monomeric subunit is formed by a β-sheet consisting of four parallel strands and one antiparallel strand. This conserved β-structure is flanked on both sides by α-helices. The C-terminal part adjacent to the β-sheet domain is mostly helical. Each monomer contains one zinc atom located in the active site at the bottom of a narrow tunnel, similarly as in ScNce103p [15].

While Δ29_CaNce103p was the only variant that successfully crystallized, the solved structure corresponds to CaNce103p lacking the first 60 amino acids.
This indicates the high flexibility of the N-terminal part of the enzyme.

**CaNce103p active site**

The active site of \( \Delta 29_{-} \text{CaNce103p} \) is formed by the catalytic \( \text{Zn}^{2+} \) coordinated by the Sy atom of Cys 164, Ne2 atom of His 160 and Sy atom of Cys 163 located 2.3 Å from the acceptor atom. According to these data, CaNce103p appears to be a member of the type I β-CAs, the active sites of which are typically formed by two cysteines, one histidine and a fourth ligand—usually water, acetic acid or acetate ion [7]. However, in the present structure, a molecule of \( \beta \)-mercaptoethanol originating from the crystallization buffer fills the fourth position (Fig. 4d).

The zinc coordination sphere is located near the dimer interface. Two of the residues contributing to the zinc coordination sphere are located at the tips of the \( \beta \)-sheets (Cys 106 at \( \beta 1 \) and His 160 at \( \beta 3 \)). Cys 163 is located outside of the \( \beta \)-sheet core. The catalytic site is surrounded by amino acids located between the \( \alpha2 \) and \( \alpha4 \) helices of the contributing monomer units (Figs. 1 and 5a). The contributing residues, most of which are hydrophobic (monomer providing zinc ion ligands: Ile 129, Gly 165; neighboring monomer: Phe 146, Leu 151), create a narrow tunnel (Fig. 4b), which serves as the only point of entry to the positively charged active site (Fig. 5d). The tunnel’s shape and openness may be regulated by the Arg 111 – Asp 163 salt bridge that also contributes to formation of the active site cavity. This salt bridge may function as a pH-dependent regulator of the catalytic activity of \( \Delta 29_{-} \text{CaNce103p} \) [21].

**Comparison of CaNce103p to other fungal carbonic anhydrases**

We aligned the crystal structure of \( \Delta 29_{-} \text{CaNce103p} \) with other known β-CA structures from *Cryptococcus neoformans* (Can2; PDB code: 2W3N), *Saccharomyces cerevisiae* (ScNce103p; PDB code: 3EYX) and *Sordaria macrospora* (CAS1; PDB code: 4O1J). The alignment revealed very high similarity among these homologs. The monomer subunits of all structures are nearly identical, although significant differences occur in the N-terminal part. Of the CAs characterized to date, ScNce103p shares the highest sequence homology with \( \Delta 29_{-} \text{CaNce103p} \) (Fig. 1). At the overall structural level, however, \( \Delta 29_{-} \text{CaNce103p} \) is more closely related to CAS1, which also forms a tetramer. The root mean square deviation (RMSD) for the superposition of 142 Ca atoms of these proteins is 0.7 Å. CAs from *S. cerevisiae* and *C. neoformans* form dimers, and RMSD values for superposition of ScNce103p and Can2 with CaNce103p are 1.2 Å for 124 Ca pairs and 1.1 Å for 132 Ca pairs, respectively.

The N-terminal part of \( \Delta 29_{-} \text{CaNce103p} \) resembles those of CAS1 and ScNce103p, while the Can2 structure includes an additional helix. However, there are similarities in the substrate tunnel region of the Can2 and \( \Delta 29_{-} \text{CaNce103p} \) structures. The substrate tunnels have similar shapes and orientations (Fig. 5b), although the middle part of the \( \Delta 29_{-} \text{CaNce103p} \) substrate tunnel is rather narrow compared to those of other fungal CAs (Fig. 5c). We observed the most pronounced differences in shape and proportion of the substrate tunnel when comparing the \( \Delta 29_{-} \text{CaNce103p} \) and ScNce103p structures, which interestingly share the highest sequence homology. The active site structure and overall structure of \( \Delta 29_{-} \text{CaNce103p} \) are nearly identical to those of CAS1.

**Discussion**

CAs are being investigated as drug targets and as potential components of carbon sequestration systems to alleviate increasing concentrations of atmospheric CO\(_2\) [2]. The fungal CA structure presented here is the sixth that has been solved to date, joining structures of CAs from *Aspergillus oryzae*, *Cryptococcus neoformans*, *Saccharomyces cerevisiae* and two CAs from *Sordaria macrospora*. The latter display the highest structural similarity to CaNce103p; one, CAS1, was used as a search model for CaNce103p structure determination. Interestingly, *S. macrospora* is the only fungus investigated to date that can survive in atmospheric air in the absence of a functional CA [8]. In *C. albicans* and *S. cerevisiae*, Nce103p is dispensable only in high CO\(_2\) concentrations.

Human CAs are structurally different from yeast CAs. β-CAs do not occur in humans, and therefore may serve as a convenient target for antifungal drugs. However, currently available yeast CA inhibitors lack sufficient selectivity. Characterizing the structure of Nce103p may lead to design of more selective and potent inhibitors, which may have applications as surface disinfectants.

Unlike the orthologous enzyme from baker’s yeast, CaNce103p was found to be a tetramer composed of two dimers. Dimerization is mediated by N-terminal helices from each subunit, formed by amino acids 66–83 and 86–94. N-terminal truncation was necessary to obtain crystals, suggesting that the start of the N-terminal domain is likely flexible and less structured. CaNce103p crystallization required removal of the first 29 residues, while truncation by 13 residues was sufficient for ScNce103p to crystallize.

The segment encompassing residues 30–48 appears to play an important role in multimerization of CaNce103p subunits, as the variant lacking the first 48 amino acids occurred only in monomeric form. However, the conformation of this segment remains unclear because the first 54 amino acids of CaNce103p were not visible in the crystal structure. Interestingly, residues 263–281, which form the end of the CaNce103p C-terminus, were not visible in our structure. The C-terminal part of CaNce103p is longer than that of the orthologous
Fig. 5 (See legend on next page.)
ScNce103p. Non-conserved, flexible termini might play a role in interactions between CAs and other molecules, calling for further investigation.

The active site of Δ29_CaNce103p does not differ substantially from those of other type I β-CAs. The only distinct feature was the molecule of β-mercaptoethanol coordinating the catalytic zinc ion. β-Mercaptoethanol was an important component of all the buffers used during Δ29_CaNce103p purification, and the presence of a reducing agent appears to be essential for the protein’s stability and solubility. The active site is located at the bottom of the substrate tunnel, the size of which differs from substrate tunnels of other structurally characterized fungal CAs.

Currently available data suggest that CAs from C. albicans and S. cerevisiae are likely to play a similar physiological role. They are also close homologs at the amino acid sequence level. On the structural level, however, ScNce103p and Δ29_CaNce103p differ.

Conclusions

In the present work, the crystal structure of N-terminally truncated CA from the pathogenic yeast Candida albicans (Δ29_CaNce103p) was determined at 2.2 Å resolution. It is the sixth fungal CA to be structurally characterized by X-ray crystallography. To obtain crystals, truncation of the 29 N-terminal amino acids was necessary. Δ29_CaNce103p forms a homotetramer organized as a dimer of dimers. Although the overall molecular architecture and the active site structure of Δ29_CaNce103p share similarities with other β-class CAs, the N- and C-terminal parts of the monomeric subunits and the substrate tunnel differ. The structure of Δ29_CaNce103p will aid the design of inhibitors that potentially could be incorporated into antimycotic surface disinfectants.

Methods

Cloning and expression of CaNce103p

Genomic DNA was isolated from C. albicans strain HE109 obtained from the mycological collection of the Faculty of Medicine, Palacky University, Olomouc, Czech Republic. The gene NCE103 and its truncated versions were amplified using the primers listed in the Table 1 and inserted using the InFusion HD Cloning Kit (Clontech) into a pET22b vector linearized with NdeI and XhoI restriction enzymes. All DNA segments resulting from PCR were verified by sequencing. The resulting expression vectors were transformed into Escherichia coli BL23(DE3). Bacteria were cultivated in Luria-Bertani medium containing 50 μg/ml ampicillin and 0.5 mM ZnSO4 in a rotation shaker at 37 °C [8]. When OD600nm reached 0.8, production of CaNce103p was induced by addition of IPTG to a final concentration of 1 mM. The culture was then incubated overnight at 20 °C, and the cells were harvested by centrifugation at 4000 g for 10 min. The cells were resuspended in 10 mM Tris-Cl, pH 8, containing 0.5 M NaCl and disintegrated using an EmulsiFlex-C3 homogenizer. The cell lysate was centrifuged at 15,000 g for 15 min at 4 °C. CaNce103p was present in the supernatant and was purified using a HiTrap Ni column (GE Healthcare) equilibrated in 10 mM Tris-Cl, pH 8, 0.5 M NaCl. Proteins were eluted with a 0–0.5 M imidazole gradient. The eluate was collected in test tubes containing 20 mM Tris-Cl, pH 8, supplemented with 40 mM β-mercaptoethanol, 400 mM NaCl and 20% glycerol. The ratio of eluate to buffer in the test tubes was 1:1. Selected fractions were pooled and dialyzed against 10 mM Tris-Cl, pH 8, containing 20 mM β-mercaptoethanol, and 10% glycerol. The final purification step was anion-exchange chromatography on a MonoQ column equilibrated in 10 mM Tris-Cl, pH 8, 20 mM β-mercaptoethanol, 10% glycerol. Proteins were eluted using a 0–1 M NaCl gradient. The efficiency of purification was analyzed using SDS-PAGE, Western blotting and activity assays. The amino acid sequences of the obtained proteins were verified by N-terminal protein sequencing and MS mass-fingerprinting analysis.

Enzyme activity

Carbonic anhydrase activity was tested using a colorimetric assay to measure CO2 hydration. Samples of purified proteins were added to final concentration of 0.005 mg/ml to 5 mM HEPES, pH 7.5, containing 20 mM Na2SO4 and 200 μM phenol red. This suspension was mixed in a 1:1 volume ratio with 3.2 mM CO2 solution using a stopped flow device (Rapid Mix accessory RX2000 from Applied Photophysics), and the reaction was monitored using a spectrophotometer (Unicam UV-510 from Thermo Spectronic).
Absorbance at 557 nm was recorded for 30 s but only the first second was used for the activity analysis. Control reactions were performed in the absence of enzyme.

Size exclusion chromatography
To determine the oligomeric state of carbonic anhydrases, size exclusion chromatography was performed using an HR200 Increase column equilibrated in 10 mM Tris-Cl, pH 8, 20 mM β-mercaptoethanol. The flow rate was adjusted to 0.5 ml/min, and the column was calibrated using the Gel Filtration Markers Kit for molecular weights 12,000–200,000 from Sigma Aldrich.

Crystallization, data collection and structure determination
CaNce103p and its truncated versions (Δ29_CaNce103p, Δ48_CaNce103p, Δ61_CaNce103p) were concentrated using an Amicon Ultra-30 ultrafiltration device (Millipore) to 10 mg/ml. Initial crystallization trials were performed with the help of a Gryphon crystallization workstation (Art Robbins Instruments) by the sitting drop vapor diffusion method at 18 °C in 96-well plates; 0.2 μl protein solution was mixed with 0.2 μl well solution and the mixture was equilibrated over a 200 μl reservoir solution. PEGs Suite I and JCSG Core I Suite (QIAGEN) were used for the crystallization condition screen. Initial microcrystals of Δ29_CaNce103p appeared in several days under the following conditions: 0.2 M ammonium acetate or 0.2 M magnesium chloride, 0.1 M Bis-Tris, pH 5.5, 25% (w/v) PEG 3350 or 45% (w/v) MPD. Further subsequent optimization of crystallization conditions involved changing to the hanging drop mode, which was performed in NeXtal plates (Qiagen) for easy crystal manipulation. Final crystals were obtained by mixing 3 μl Δ29_CaNce103p with 1 μl reservoir solution composed of 0.1 M ammonium acetate, pH 5.5, 25% PEG 3350.

For data collection, the crystal was frozen in liquid nitrogen. For cryoprotection, the crystals were soaked for 10 s in the corresponding reservoir solution supplemented with 25% (v/v) glycerol. Diffraction data for

Table 1 Primers used in this study

| Construct | Primer name | Sequence | Restriction site |
|-----------|-------------|---------|-----------------|
| WT        | WT_CaNCE103 Nde I F | AAGGAGATATACATATGATGGGTAGAGAAAATATTTTGAA | Nde |
| Δ29       | Δ29_CaNCE103 Nde I F | AAGGAGATATACATATGATGGGTAGAGAAAATATTTTGAA | Nde |
| Δ29/Δ48/Δ61_CaNCE103 Xho I R | GGTGGTGGTGGTGCTCGAGTCAATGAGGGTTATATTCTTCTTC | Xho |
| Δ48       | Δ48_CaNCE103 Nde I F | AAGGAGATATACATATGATGGGTAGAGAAAATATTTTGAA | Nde |
| Δ29/Δ48/Δ61_CaNCE103 Xho I R | GGTGGTGGTGGTGCTCGAGTCAATGAGGGTTATATTCTTCTTC | Xho |
| Δ61       | Δ61_CaNCE103 Nde I F | AAGGAGATATACATATGATGGGTAGAGAAAATATTTTGAA | Nde |
| Δ29/Δ48/Δ61_CaNCE103 Xho I R | GGTGGTGGTGGTGCTCGAGTCAATGAGGGTTATATTCTTCTTC | Xho |

Table 2 Crystal data and diffraction data collection and refinement statistics

| Data-collection statistics | |
|---------------------------|------------------|
| Wavelength (Å)            | 0.9184           |
| Space group               | P2₁ 2₁ 2₁        |
| Unit-cell parameters (Å, °) | a = 69.37, b = 90.29, c = 167.12, α = 90.0, β = 90.0, γ = 90.0 |
| No. of molecules in asymmetric unit | 4 |
| Resolution range (Å)      | 50–2.2 (2.33–2.20) |
| No. of unique reflections  | 53,038 (8439)    |
| Multiplicity               | 4.5 (4.6)        |
| Completeness (%)           | 98.1 (98.2)      |
| Rmerge†                    | 16.5 (238.5)     |
| Average I/σ(I)             | 8.27 (0.62)      |
| Wilson B (Å²)              | 49.0             |
| Refinement statistics      |                   |
| Resolution range (Å)      | 45.95–2.20 (2.3–2.20) |
| No. of reflections in working set | 51,601 (6327) |
| No. of reflections in test set | 1053 (129) |
| Rwork‡                     | 24.1 (40.5)      |
| Rfree§                     | 27.9 (43.0)      |
| Rall (%)                   | 24.1             |
| R.m.s.d., bond lengths (Å) | 0.019            |
| R.m.s.d., bond angles (Å)  | 1.57             |
| No. of non-H atoms in asymmetric unit | 6269 |
| No. of water molecules in asymmetric unit | 35 |
| Mean ADP (Å²)              | 81.6             |
| Main chain (A/B/C/D)       | 76.5/75.8/92.0/82.2 |
| Side chain (A/B/C/D)       | 80.8/80.3/93.7/86.4 |
| Water                      | 62.3             |
| Residues in alternative conformations | 0 |
| Ramachandran plot statistics |                   |
| Residues in favoured regions (%) | 95.7 |
| Residues in allowed regions (%) | 4.1 |
Δ29_CaNce103p were collected to 2.2 Å resolution at 100 K using the MX14.2 beamline at BESSY, Berlin, Germany [22]. Diffraction data were processed using the XDS suite [23, 24] using XDSAPP2.0 [25].

The structure was determined by molecular replacement using the program Molrep. β-CA from Sordaria macrospora (PDB ID: 4O1J) was used as the search model. Model refinement was carried out using the program Phenix.refine [26] from the Phenix package (version 1.9–1692) [27], and the final cycles were performed with REFMAC 5.2 from the CCP4 package. Manual building was performed using Coot. The quality of the final model was validated with the Molprobity server.

Crystal parameters, data collection and refinement statistics are summarized in Table 2. Structural representations were prepared with the program PyMOL. Atomic coordinates and experimental structure factors have been deposited in the Protein Data Bank under code 6GWU.

Abbreviations
Can2: Carbonic anhydrase from Cryptococcus neoformans; CaNce103p: Carbonic anhydrase from Candida albicans; Cas: Carbonic anhydrases; CAS1: Carbonic anhydrase from Sordaria macrospora; NCE: Non-classical export; RMSD: Root mean square deviation; ScNce103p: Carbonic anhydrase from Saccharomyces cerevisiae

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Availability of data and materials
Crystal parameters, data collection and refinement statistics are summarized in Table 2. Structural representations were prepared with the program PyMOL. Atomic coordinates and experimental structure factors have been deposited in the Protein Data Bank under code 6GWU.

Authors’ contributions
JD conceived and performed most of the experimental work. JB1 performed data collection, data processing, structure determination, refinement and structure function analysis of CaNce103p. JB2 participated in cloning and protein purification. SM participated in protein purification, protein crystallization and activity assays. OH helped prepare one of the vectors, helped in protein purification. IP participated in crystallization and activity assays. JD conceived and performed most of the experimental work. JB1 performed deposition in the Protein Data Bank under code 6GWU. Atomic coordinates and experimental structure factors have been deposited in the Protein Data Bank under code 6GWU.

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Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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