Phytochemistry 91 (2013) 88–92

Contents lists available at SciVerse ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

High speed X-ray analysis of plant enzymes at room temperature

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ARTICLE INFO

Article history:
Available online 14 June 2012

This manuscript is dedicated to memory of late Professor Meinhart H. Zenk (Zenk Memorial issue of Phytochemistry).

Keywords:
Rauvolfia serpentina (L.) Benth
Apocynaceae
Raucaffricine-*O*-β-D-glucosidase
Enzyme crystal structures
Ligand complexes
X-ray diffraction
Room temperature
High speed measurements

ABSTRACT

X-ray measurements at room temperature (295 K) deliver high quality data sets with unprecedented speed (<2 min), as shown for crystallized raucaffricine-*O*-β-D-glucosidase (RG), its mutant RG–Glu186Gln and several ligand complexes of the enzyme which participates in alkaloid biosynthesis in the plant *Rauvolfia*. The data obtained are compared with data sets measured under typical cryo conditions (100 K). Under both conditions, density maps are highly comparable and favor the described protocol for room temperature measurements, potentially paving the way for future crystallographic studies capturing biosynthetic pathway intermediates.

1. Introduction

In recent decades, cryo cooling of enzyme and protein crystals has become a routinely used method for X-ray data collection and macromolecular 3D-structural analysis. These conditions allow minimization of significant radiation-induced damage, although the principal drawback is in the employment of ionizing X-radiation in macromolecular crystallography. However, crystals can frequently become disordered during freezing. For crystallographic studies of, for example, viruses, freezing of crystals can be problematic, and cryo conditions can cause high mosaicity (Fry et al., 1999), which may in turn compromise structural determination. Moreover, identifying a suitable cryoprotectant may be a time-consuming process. Such technical drawbacks might be overcome by development of high speed data collection at room temperature (RT). In principle, the method has been demonstrated during the last 5 years for its efficiency (Southworth-Davies et al., 2007; Rajendran et al., 2011) and ease of handling (Kalinin et al., 2005), and is expected to play an important role in plant enzymes structural biology studies in the near future.

To our knowledge, the work presented here describes the novel application of RT data collection to the 3D-structural determination of a plant enzyme, its inactive mutant and its corresponding enzyme-ligand complexes in the field of natural product biosynthesis. The results obtained for the enzyme raucaffricine-*O*-β-D-glucosidase (RG, EC 3.2.1.125), an enzyme involved in the biosynthesis of plant indole alkaloids (Scheme 1), demonstrate that, compared to X-ray measurements performed under cryo conditions, RT measurements can also be easily and routinely used in structural enzyme analysis if high speed detectors are used.

2. Results and discussion

Here it was determined and analyzed the X-ray data of RG–WT (wild type), its mutant Glu186Gln and their ligand complexes with glycerol, glucose and the terpenoid glucoside secologanin for two reasons: (i) to evaluate comparatively the quality of data measured at 100 and 295 K (Tables 1 and S1) respectively and (ii) to delineate the sugar binding pocket of RG.

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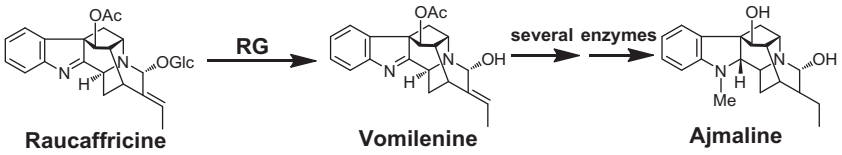
http://dx.doi.org/10.1016/j.phytochem.2012.05.009



Scheme 1. Enzymatic transformation of the glucoalkaloid raucaffricine to vomilenine catalyzed by raucaffricine glucosidase (RG) in the biosynthesis of the antiarrhythmic alkaloid ajmaline of the Indian medicinal plant *Rauvolfia serpentina* (L.) Benth.

Table 1
Structural alignment of three RG data sets collected under both cryo and room temperature conditions, respectively. All proteins used for crystallization were N-His_6-tagged enzymes. The root mean square deviation (RMSD) values were given by comparing all the data with chain A of RG–WT data (WT, wild type).

Measurement temperature	Data set	PDB code [Chain ID][c]	RMSD (Å)	Cα (compared)
100 K (Mar CCD detector)	RG–WT[a,b]	4ATD[A]	0	470
	RG–WT–glucose complex[a]	4ATL[A]	0.171	446
	RG–WT–glycerol complex	4A3Y[A]	0.071	440
295 K (Pilatus 6 M detector)	RG–Glu186Gln	3U5U[A]	0.251	438
	RG–Glu186Gln–glucose complex[a]	4EK7[B]	0.223	434
	RG–Glu186Gln–secologanin complex	3U5Y[A]	0.255	442

[a] Full 3D structures are published here the first time despite preliminary data for RG–WT (Ruppert et al., 2006).
[b] Soaked with secologanin but density for this ligand was not observed.
[c] The PDB chain ID listed in the table were used to generate the figures.

2.1. Comparison of data collected at cryo and RT conditions

All the data were aligned with RG–WT data (Table 1). The root mean square deviation (RMSD) values clearly demonstrate an excellent conformity (⩽0.27 Å). When cryo data and RT data were compared separately, the structural deviation among RG–RT data (RMSD values) was less than that observed for the cryo data, which suggests that measurements under RT conditions provide excellent reproducibility in terms of results.

The overall structure of all six cryo and RT data sets of RG are very well correlated, as illustrated by the overall structure comparison of the backbone ribbon diagrams (Fig. 1 and Table 1). Density maps of RG–WT, its complex with glucose acquired at cryo condition and RG–Glu186Gln mutant, its complex with glucose obtained at RT were further investigated (Fig. 2 and Fig. 3). As shown in Fig. 2, residues forming part of the binding pocket of RG active-site exhibit near identical density map quality regardless of the binding of glucose. For the glucose part, it is obvious that the glucose conformation is consistent with each other, except for their direction of the *O*-6 hydroxyl group. This is probably due to the fact that this hydroxyl group is highly flexible and may form a hydrogen bond with the corresponding residue in various conformations.

The equipment used for RT measurements together with the technique described offers the opportunity for high quality data collection with high resolution (Fig. 2 and Table S1) and unprecedented speed, greatly helping in the minimization of radiation damage to the crystals. Furthermore, the usually tedious mounting of crystals with glass capillaries has now been rendered as easy as the normal mounting of frozen crystals.

Researchers have also tried to improve the mounting method for RT data collection, like the one demonstrated by Kalinin et al. (2005). The free-mounting system for protein crystals with accurate adjustment of humidity at crystals demonstrated by Kiefersauer et al. (2000) was also successfully applied to RT protein crystals measurement (Fraser et al., 2009). These findings might also guide future data collection protocols and could contribute to a widespread application of X-ray structure elucidation of macromolecules at RT.

2.2. Characterization of RG glucose binding site

In addition to the above results, 3D structures of RG complexes with glycerol, glucose and secologanin also allowed for the first time detailed description of the glucoside binding site of the enzyme. The complex with glycerol was unintentionally formed as glycerol was used as cryoprotecting additive in order to avoid ice formation during freezing of the mounted crystals. Under such circumstances, glucose-recognizing enzymes and cryoprotectant

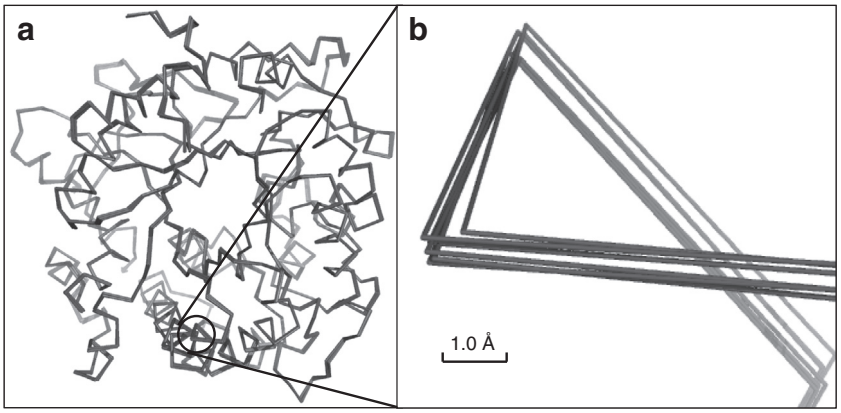


Fig. 1. (a) Structural comparison of the overall architecture by ribbon diagrams of six RG data sets in Table 1 (RG–WT, RG–WT–glucose complex, RG–WT–glycerol complex, RG–Glu186Gln, RG–Glu186Gln–glucose complex and RG–Glu186Gln–secologanin complex), measured under both cryo and room temperature condition; (b) illustrates a zoomed region from (a) in order to show the excellent agreement of data measured at room and cryogenic conditions.