



Thermal stability and enzymatic activity of quinoïn, a type 1 ribosome inactivating protein (RIP) from quinoa seeds

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Abstract

Quinoïn is a type 1 ribosome inactivating protein (RIP) isolated from *Chenopodium quinoa* seeds exhibiting enzymatic features, such as polynucleotide:adenosine glycosylase (PNAG) activity on DNA and a broad spectrum of biological functions, including antiviral, antifungal, and antitumour activities. In this study, we investigated the effects of pH and ionic strength on the enzymatic activity and thermal stability of quinoïn to better understand its functional versatility and potential for biotechnological applications. We found that quinoïn PNAG activity was enhanced under acidic conditions and in the presence of physiological salt concentration. To provide a link between its activity and structural stability, we performed a thorough biophysical analysis using circular dichroism (CD) and differential scanning calorimetry (DSC). Our data revealed that: i) quinoïn is well-folded and thermally stable over the pH range from 7.0 to 3.0; and ii) thermal stability is influenced by pH and ionic strength, pointed out at the role of charge–charge interactions. These results suggest that the enhanced PNAG activity under acidic and high ionic strength conditions is due to the increase in structural dynamics and modulation of the charge interactions in the active site. Overall, this study provides new insights into the structure–activity relationship of quinoïn and highlights its potential to operate beyond physiological pH with important biological and biotechnological applications.

Keywords *Chenopodium quinoa* · Quinoïn · Ribosome inactivating proteins · DSC · Conformational stability

Introduction

Quinoïn is a RNA N- β -glycosylase (EC: 3.2.2.22), isolated from *Chenopodium quinoa* L. seeds, which depurinates a specific adenine in the conserved Sarcin/Ricin loop (SRL) of 28 S ribosomal RNA (rRNA), leading to protein synthesis

inhibition and subsequent cell death through the apoptotic pathway [1]. In the light of this, this enzyme is classified as type 1 ribosome inactivating protein (RIP) [2]. Like most RIPs, quinoïn also exhibits polynucleotide:adenosine glycosylases (PNAG) activity, enabling it to remove adenines from poly (A), RNA, and DNA substrates [3–5].

RIPs are a group of well-characterized toxins classically divided into three types: i) type 1 (such as quinoïn), which are much less cytotoxic due to the absence of the B lectin chain; ii) type 2, which differ from type 1 RIPs by the presence of the B chain and their ability to enter cells [6]; and iii) type 3, also named atypical type 1 RIPs, which are synthesized as proenzymes and activated only after the removal of a short internal peptide segment. Most RIPs are produced by flowering plants (*Angiospermae*), although ribosome inactivating and protein synthesis-inhibitory proteins or peptides have also been identified in mushrooms, bacteria (bacterial RIPs), mosquitos (metazoan RIPs), and even in a brown alga (*Laminaria japonica*) [7]. The physiological role is still not fully understood, although recent experimental evidence highlights their

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role in defence mechanisms against biotic [8] and abiotic stresses [9].

Quinoin is a monomeric glycoprotein (~29-kDa) exhibiting other enzymatic features common to type 1 RIPs, such as PNAG activity on salmon DNA and endonuclease activity on supercoiled plasmid DNA [1, 5]. In addition, our structural studies revealed that quinoin, as reported for all known RIPs [10], consists of two domains, with an active cleft located at their interface [11] and containing two conserved catalytic residues (Glu 177 and Arg180; numbering refers to the quinoin amino acid sequence), and four residues involved in the binding with adenine (Tyr75, Tyr122, Phe181, and Trp206). Furthermore, this enzyme possesses a broad spectrum of biological and antipathogenic action such as antiviral, antifungal, and antitumour activities. Indeed, recent studies have highlighted that quinoin exerts: i) a cytotoxic effect against human fibroblasts (BJ-5ta) and human keratinocytes (HaCaT) [1] as well as on human continuous and primary glioblastoma cell lines [12]; ii) an antiviral activity towards tobacco necrosis virus (TNV) [11] and SARS-CoV-2, altering viral replication in the latter case [13]; iii) an antifungal activity against phytopathogenic fungi (e.g. *Botrytis cinerea* and *Trichoderma harzianum*) [14]; and iv) ROS-mediated genotoxicity effect towards the zebrafish genome [15]. In addition, considering that RIPs have long been thought of as potentially perfect components in immunotoxins and nanoconjugates for the treatment of human tumours [4, 16, 17], quinoin was chemically conjugated with cetuximab antibody in order to obtain a selective immunotoxin against the cetuximab-resistant GEO-CR cells. The novel quinoin cetuximab immunoconjugate exhibits a significant dose-dependent cytotoxicity towards GEO-CR cells [18]. These findings, together with similar results reported for other edible plant RIPs such as trichosanthin (TCS; from *Trichosanthes kirilowii* Maxim.) [19] and *Momordica charantia* lectin (MCL; isolated from *Momordica charantia* L.) [20, 21], emphasize the potential biotechnological applications of quinoin as an antiviral, antifungal, and anticancer agent.

Quinoin and RIPs, which are harmful to the same cells that synthesize them, are generally synthesized as preforms and then released either into the extracellular space (pH variable in the presence of stress) or into vacuoles (pH ~5.0) [22, 23], which have a different pH compared to the cytosol (pH ~7.0) [24]. Indeed, pH value is known to play a critical role in plant immunity, abiotic stress response, and plant growth and development [25]. In addition, pH changes induced by plant–pathogen interactions regulate the growth–defence balance in plants [26]. Therefore, understanding the influence of pH variation on the enzymatic activity or biological role of quinoin could provide new strategies for its usefulness in plant disease control and biomedicine.

In this context, considering the potential applications of quinoin in the biotechnological and biomedical fields, we present the enzymatic and physicochemical characterization of quinoin at different pH values, both in the absence and in the presence of NaCl 150 mM (physiological concentration).

Interestingly, we found that the enzymatic PNAG activity of the enzyme is improved in acidic conditions. In addition, physicochemical results clearly showed that quinoin is well-folded and thermally stable over the wide pH range from 7.0 to 3.0. Overall, our findings contribute to a better understanding of this versatile protein and support its potential use as a novel biotechnological tool.

Materials and methods

Materials

Chemicals (analytical grade, e.g. buffers and salts) and single-stranded DNA from salmon testes (product number: D7656) were obtained from Sigma-Aldrich Solutions (Merk Life Science, Milan, Italy).

Quinoin purification

Quinoin was purified as previously described by Landi *et al.* [1] from *C. quinoa* seeds using a general protocol for the preparation of type 1 RIPs [3, 27]. The purified enzyme was pooled, dialyzed against water, freeze-dried, and stored at −20 °C until use.

Spectrophotometric procedures

UV spectra of DNA were recorded with a Cary-100 double-beam UV–Vis spectrophotometer (Agilent Technologies Italia S.p.A., Cernusco s/N, Milan, Italy). Briefly, 25 µg of single-stranded DNA (ssDNA) from salmon testes were added to a final DNA concentration of 1.0 mg mL^{−1} in 10 mM buffer at different pH values (Na-phosphate at pH 7.0 or pH 6.0, Na-acetate at pH 5.0 or pH 4.0, or glycine–HCl at pH 3.0), as well as in the presence of NaCl 150 mM. The UV spectral data were measured in the spectrum acquire mode at a wavelength range of 220–320 nm, and a re-plot of absorbance against wavelength of all spectra recorded at the same pH was performed by using Prism, version 8 (GraphPad Software Inc., San Diego, CA, USA). All spectra were performed in triplicate.

Polynucleotide:adenosine glycosylase (PNAG) activity on salmon sperm DNA

PNAG activity was assayed on single-stranded DNA (ssDNA) from salmon testes (Sigma, Milan, Italy). Equal amounts of quinoïn [0.1 nmole (~3.0 µg; 0.34 µM)] were incubated with 100 µg of ssDNA (final concentration 0.33 mg mL⁻¹) at 30 °C for 1 h in 10 mM buffer at different pH values (Na-phosphate at pH 7.0 or pH 6.0, Na-acetate at pH 5.0 or pH 4.0, or glycine–HCl at pH 3.0), as well as in the presence of 150 mM NaCl (final volume 0.3 mL). The enzyme:substrate ratio was about 1:33; v:v. Following incubation, DNA was precipitated overnight with 2.5 volume cold ethanol in the presence of Na-acetate 0.14 M pH 5.2 (0.1 volume) at –80 °C and centrifuged at 18,000 *g* for 30 min at 4.0 °C. Adenine released was determined in the supernatants spectrophotometrically at 260 nm. The same amount of BSA (3.0 µg) was used as a negative control. All analyses were performed in triplicate.

Circular dichroism

Circular dichroism (CD) measurements were taken using a Jasco J-1500 spectropolarimeter (Jasco—Japan) equipped with a Peltier temperature control system. Far-UV CD spectra were recorded at a fixed temperature of 25 °C over the 190–260-nm wavelength range. A quartz cuvette (Hellma, Plainville, NY, USA) with a 0.1 cm optical path length was used for all measurements under the following experimental conditions: 0.5 nm resolution, 50 nm/min scan speed, 4 nm bandwidth, and 2 s integration time. Final spectra represent the accumulation of three scans. Protein samples were prepared at a concentration 0.1 mg/mL after dialysis in 10 mM buffer solutions, including sodium phosphate (pH 7.0), sodium acetate (pH 5.0 and 4.0), and glycine–HCl (pH 3.0). Background signals were subtracted from all samples. The mean residue ellipticity, $[\theta]$ in deg cm² dmol⁻¹, was calculated from the equation: $[\theta] = [\theta]_{\text{obs}} \cdot \text{MRW} \cdot (10 \cdot l \cdot c)^{-1}$ where $[\theta]_{\text{obs}}$ is the ellipticity measured in degrees, MRW is the mean residue molecular mass (Da), *c* is the protein concentration in g L⁻¹, and *l* is the optical path length of the cell expressed in cm. The secondary structure content of quinoïn was estimated by deconvoluting the CD spectra using the local version of DichroWeb (DichroWebGit) [28], applying the algorithm SELCON3 with the reference dataset SP175.

Differential scanning calorimetry

DSC measurements were taken by means of Nano DSC (TA Instruments, New Castle, DE, USA) equipped with 300-µL twin gold capillary cells under 3 atm pressure. The partial molar heat capacity of the protein in solution was measured as a function of temperature, and the excess heat capacity

function ($\langle \Delta C_p \rangle$) was obtained after baseline subtraction. Buffer–buffer scans were recorded under the same conditions and subtracted from sample profiles. The reversibility of thermal unfolding was verified by reheating criterion: a reheating scan after cooling must be superimposable to the first heating. A scan rate of 1.0 °C/min in the temperature range of 25–85 °C was chosen for all the experiments. Protein samples were prepared at a concentration of 0.5 mg/mL after dialysis in 10 mM buffer solutions, including Na-phosphate (pH 7.0), Na-acetate (pH 5.0, pH 4.0), and glycine–HCl (pH 3.0). Data analysis was performed by using the Nano Analyze software, provided by the manufacturer and plotted using the Origin software package (OriginLab, Northampton, MA, USA). The melting temperature, T_m , was measured by the midpoint of the DSC curve while the molar enthalpy, ΔH^{cal} , by integrating the area under the transition peak. The van't Hoff enthalpy, ΔH^{vH} , is calculated according to the following equation [29]:

$$\Delta H^{\text{vH}} = \frac{4RT_m^2 C_{p, \text{max}}}{\Delta H^{\text{cal}}} \quad (1)$$

where $C_{p, \text{max}}$ is the maximal molar excess heat capacity measured at T_m , and *R* is the gas constant (8.314 J mol⁻¹K⁻¹). The $\Delta H^{\text{vH}}/\Delta H^{\text{cal}}$ ratio allows for the discrimination of different unfolding models. Specifically, when $\Delta H^{\text{vH}}/\Delta H^{\text{cal}} = 1$, the protein unfolding is well described by a two-state model, suggesting that the protein has a single energetic domain. When $\Delta H^{\text{vH}}/\Delta H^{\text{cal}} < 1$, the DSC profile suggests partially overlapping transitions, with the protein unfolding according to a non-two-state model [30].

Results and discussion

Evaluation of quinoïn PNAG activity at different pHs

Quinoïn is a N-β-glycosylase (EC: 3.2.2.22) which breaks the N-glycosidic bond of a specific adenine in the SRL of the major rRNA (A₄₃₂₄, rat liver 28S rRNA) [1, 31]. It also possesses PNAG activity, i.e. the ability to remove adenine from polynucleotide substrates (e.g. poly (A) and viral RNA and DNA) [1, 32]. Both enzymatic actions are necessary for the biological activity of quinoïn (e.g. cytotoxicity and antiviral activity) [13, 18].

The release of the β-fragment (hallmark of RIPs) resulting from the specific N-β-glycosylase action of quinoïn on ribosomes can be detected by Endo's assay, a biochemical method, which must be performed under strictly physiological conditions to preserve the ribosome integrity [31, 33–35]. Consequently, this assay cannot be applied to monitor enzymatic activity under different buffers, pHs, and/or ionic strength conditions [36–38]. However, these

parameters can change *in vivo* and influence RIPs function, depending on cellular localization (apoplast, vacuole, or cytosol) [39–41] or in response to abiotic (e.g. temperature, drought and salinity [9, 42]) and biotic stress [8, 43]. To address this Endo's assay limitation, we evaluated the PNAG activity of quinoïn, which can be monitored under various experimental conditions [32], including different pH values and/or ionic strengths [44–46].

The PNAG activity of quinoïn was measured in the absence (Fig. 1A) or presence of NaCl 150 mM (Fig. 1B) at different tested pHs. In both conditions, quinoïn exhibited the PNAG activity at acidic pH; however, a different behaviour is evident with and without NaCl. Indeed, in buffer alone, quinoïn showed higher PNAG activity at pH 4.0, which decreased to 79.0% and 38.7% at pH 3.0 and pH 5.0, respectively, as well as 85.4% and 96.2% at pH 6.0 and pH 7.0, respectively. Conversely, in the presence of 150 mM NaCl, the highest PNAG activity of quinoïn was detected at pH 3.0 with an increase of 14.1% with respect to pH 4.0 in the same conditions, while the PNAG activity decrease of 84.7%, 90.8%, and 91.6% at higher pH (pH 5.0, 6.0, and 7.0, respectively). Thus, in the presence of salt, the PNAG activity of quinoïn is 1.14-fold higher at pH 3.0 with respect to pH 4.0.

These data highlight that the PNAG activity of quinoïn is pH and ionic strength dependent, suggesting a possible different biological action of quinoïn during abiotic and biotic stress in plants. On the other hand, considering that the substrate could undergo structural rearrangements under the conditions tested, a spectrometric analysis of ssDNA used as a substrate was carried out (Fig. S1). The superimposed spectra of the oligonucleotide under different ionic strength conditions at the selected pHs showed that the presence

of NaCl caused a decrease in the absorbance at 260 nm (see Fig. S1 panel E), indicative likely of DNA conformational rearrangement (e.g. compaction) [44, 46], promoting enzyme–substrate interaction. Indeed, whenever there is a decrease in absorption at 260 nm, there is a relative increase in quinoïn PNAG activity.

Overall, considering that the variation of the PNAG activity of quinoïn at different pHs with and without NaCl could be partly justified by the rearrangements in the ssDNA substrate, we decided to evaluate the possible structural changes of the quinoïn enzyme under the tested conditions by carrying out a complementary physicochemical characterization.

Circular dichroism

First, to evaluate the impact of pH on the secondary structure of quinoïn, we recorded far-UV CD spectra in appropriate buffer solutions. The results are collected in Fig. 2.

CD spectra of quinoïn show two minima at around 208 and 222 nm, with a positive band rising below 200 nm, indicating that this protein adopts a well-defined secondary structure. Interestingly, we did not observe any remarkable change in the spectral features upon varying the solution pH, revealing that the overall secondary structure is rather insensitive to alterations in the ionization state of charged amino acids. To obtain more details on quinoïn secondary structure content, we performed a deconvolution analysis using the tool Dichroweb [28]. The results are reported in Table 1 and compared with the values obtained from the analysis of a three-dimensional structural model of the protein predicted with the tool AlphaFold3 [47].

The deconvolution analysis performed on the experimental data reveals that quinoïn presents both α - and β -structures

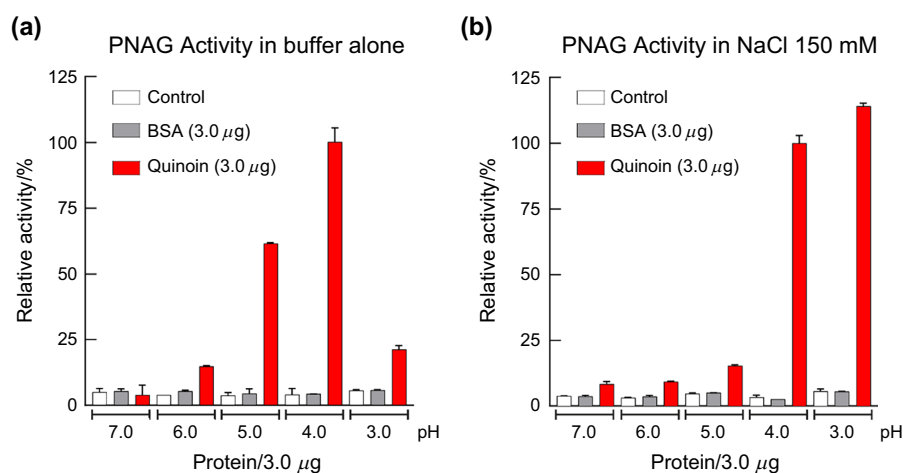


Fig. 1 Polynucleotide:adenosine glycosylase (PNGA) activity of BSA (negative controls) or quinoïn assayed on ssDNA. PNAG activity was measured in the absence (A) or presence of NaCl 150 mM (B) at different tested pHs. Values on the y-axis are expressed relative to the

maximum activity observed at pH 4.0 with or without NaCl. Proteins (3.0 μg) were assayed on salmon sperm DNA as described in materials and methods. Data represent the mean of two biological replicates each including three technical replicates $\pm$ SD.

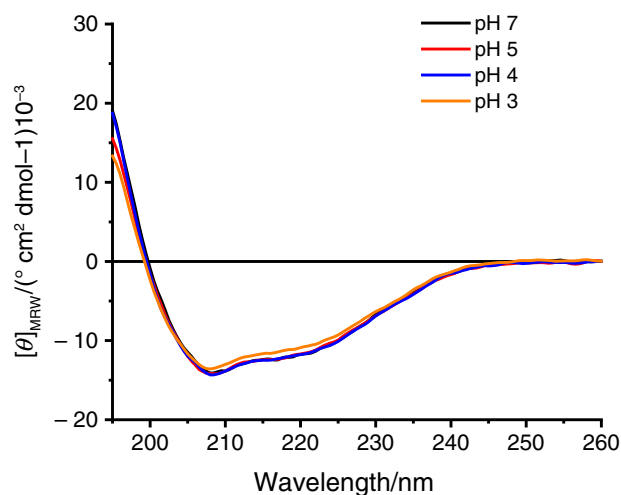


Fig. 2 Far-UV CD spectra of quinoïn in the selected buffer solutions at the indicated pH values and 25 °C. The protein concentration was 0.1 mg/mL.

Table 1 Results of the deconvolution analysis performed on the CD spectrum of quinoïn at pH 7.0 with DichroWebGit, using the reference dataset SP175, and secondary structures content obtained from the analysis of quinoïn AlphaFold3 model.

Tool	% α -helix	% β -sheet	%random ^[b]
DichroWebGit ^[a]	33	21	46
AlphaFold3	39	20	40

[a] NRSMD value is below 0.1; [b] it includes turns

with values, which are consistent with those calculated from the AlphaFold3 model, suggesting that the prediction is a good approximation of the real protein structure.

Differential scanning calorimetry

To evaluate the dependence of protein thermal stability on pH, differential scanning calorimetry (DSC) experiments in the appropriate buffers were performed. Additionally, for each pH condition, we repeated the experiment in the presence of a physiological salt concentration (150 mM NaCl) to assess the impact of ionic strength on protein denaturation. The obtained calorimetric curves are collected in Fig. 3, and the corresponding thermodynamic parameters are reported in Table 2.

DSC results revealed that quinoïn exhibits high thermal stability throughout the entire pH range examined. In fact, the unfolding enthalpy (ΔH^{cal}) ranged from 930 kJ/mol at pH 7.0 to 730 kJ/mol at pH 3.0, values which are notably higher compared to those reported for proteins of similar size [42]. Consistently, the melting temperature (T_m) was 72.6 °C, at pH 7.0 and increased up to 74.5 °C and 73.6 °C, at pH 5.0 and 4.0, respectively. However, upon further decreasing the

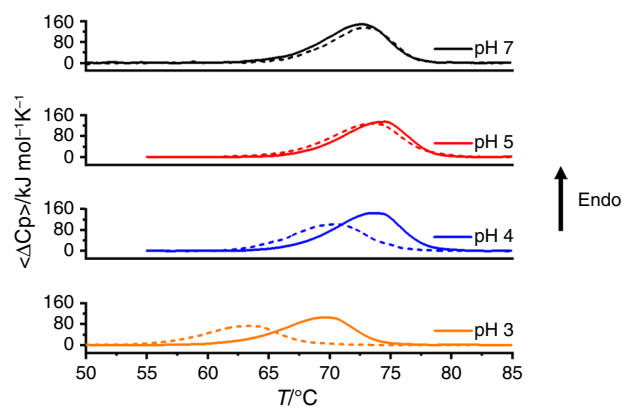


Fig. 3 Representative DSC curves from three independent experiments of quinoïn in the selected buffer solution at the indicated pH values in the absence (solid lines) and presence (dashed lines) of 150 mM NaCl.

Table 2 Thermodynamic parameters obtained by DSC curves analysis.

pH	Neat buffer		+ 150 mM NaCl	
	ΔH^{cal} /kJ/mol	T_m /°C	ΔH^{cal} /kJ/mol	T_m /°C
7.0	930	72.6	870	72.9
5.0	900	74.5	790	74.5
4.0	800	73.6	760	73.6
3.0	730	69.6	580	63.3

Errors on melting temperature and enthalpy are within ± 0.5 °C and 8% of the reported values. Each value represents the average of at least three independent DSC measurements.

pH (pH 3), the T_m dropped to 69.5 °C, indicating that quinoïn achieves its maximum thermal stability between pH 4.0 and 5.0. This behaviour can be explained considering the variation of the ionization state of key protein residues as a function of pH. For example, His90, the sole histidine in the sequence, becomes protonated at pH values below 6.0. According to the AlphaFold3 model, when protonated, this residue can form electrostatic interactions with Asp81 in a buried region, contributing to the stabilization of the protein core (see Fig. 4). However, if the pH is further reduced, the additional protonation of Asp and Glu residues increases the protein's overall positive charge (quinoïn isoelectric point is at pH 9.3), disrupting salt bridges and enhancing repulsive interactions between similarly charged segments. The reduction and weakening of stabilizing contacts in the tertiary structure can ultimately increase protein flexibility.

The strong pH-dependence of the T_m highlights the importance of charge–charge interactions in the stabilization of quinoïn structure. To confirm this, we repeated DSC experiments in the presence of a physiologically relevant salt concentration (150 mM NaCl). At all the pHs explored

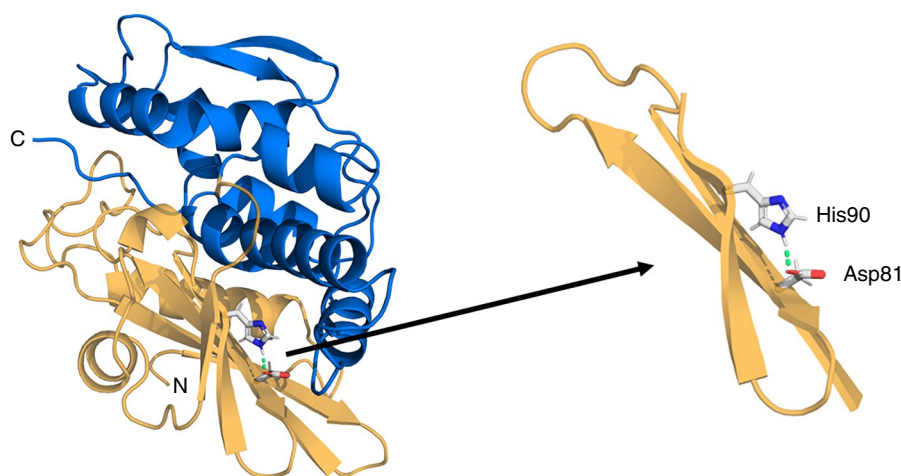


Fig. 4 Three-dimensional molecular model for quinoine obtained with AlphaFold3 [47]. The structure was visualized with the software PyMol 3.1.0 [50]. The protein backbone is shown as a cartoon, His90, and Asp81 sidechains are reported as sticks. The N-terminal

domain is bright orange (residues 1–119), and the C-terminal domain is blue (residues 120–254). A focus on His90 and Asp81 is reported to highlight the potential of these two residues to establish a salt bridge (short dashed in green) when His90 is protonated.

except 7.0, both T_m and ΔH^{cal} decreased, consistent with a salt-induced screening of electrostatic interactions. This effect was more dramatic at pH 3, where fewer stabilizing interactions are present and a loss of structural rigidity is expected. This enhanced protein flexibility may promote a better fit of the active site to the substrate, improving recognition and, in turn, quinoine PNAG activity (see Fig. 1). While pH affects both the structure of the ssDNA substrate and quinoine, additional factors must be considered, particularly when comparing enzymatic activity at pH 4.0 and pH 3.0. Type 1 RIPs like quinoine contain key charged residues, Glu177 and Arg180, in the active cleft. While Arg180 is protonated across all the conditions explored, Glu177, proposed to stabilize the oxocarbenium ion intermediate [48, 49], may become protonated as the pH decreases from 4.0 to 3.0. Glu177 protonation could thus impair its ability to stabilize the cationic intermediate, reducing the catalytic activity. However, the increase of ionic strength at low pH may compensate via charge shielding, restoring the catalytic activity (see Fig. 1 panel B).

To note that the thermal unfolding of quinoine is fully irreversible under all the investigated pH conditions, except for pH 3.0, irreversible thermal unfolding has been reported for other type I RIP family members, such as saponin S6 and gelonin, for which non-reversible aggregation steps were identified during thermal denaturation at neutral pH using FTIR spectroscopy [51, 52].

The observed thermal irreversibility may arise from the exposure and association of hydrophobic regions of the protein, which are not entirely counterbalanced by electrostatic charge–charge repulsion. This balance appears to be achieved at pH 3.0, where the thermal unfolding of quinoine can be considered a reversible process. In fact,

according to the reheating criterion, for the second heating after cooling, we measured an enthalpy value of 620 kJ/mol with an enthalpy recovery of 85% (Fig. S2). To analyze the unfolding mechanism of quinoine in more detail, we used the data obtained at pH 3.0 reported in Fig. 5A where the process is reversible. To propose a model for the quinoine unfolding, we applied the van't Hoff test based on DSC experimental data [43]. The van't Hoff enthalpy of quinoine, $\Delta H^{vH} = 550 \text{ kJ mol}^{-1}$ was calculated for the thermal profile at pH 3.0 using Eq. (1) and yielded a $\Delta H^{vH}/\Delta H^{cal}$ ratio of 0.75, suggesting that quinoine unfolding occurs via a non-two-state mechanism involving at least two overlapping transitions. Therefore, we performed a deconvolution analysis of quinoine DSC profile. The result is reported in Fig. 5B, and the corresponding parameters are collected in Table 3.

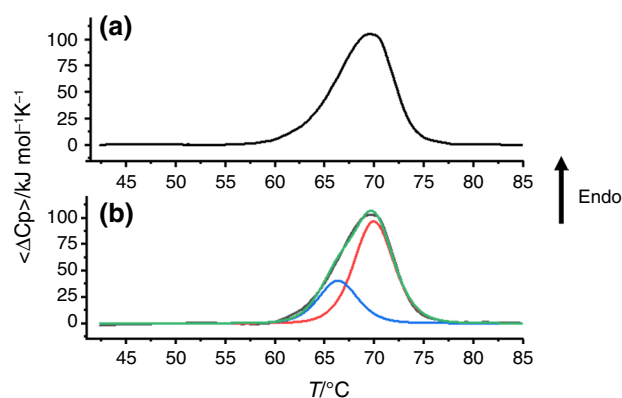


Fig. 5 **A** Representative DSC profile from three independent experiments of quinoine in buffer solution at pH 3.0. **B** Deconvolution of DSC profile of quinoine in buffer solution at pH 3.0 according to the non-two-state model.

Table 3 Experimental and calculated thermodynamic parameters obtained from data analysis and deconvolution of thermal profile for quinoïn in buffer solution at pH 3.0.

$T_m/^\circ\text{C}$	$\Delta H^{\text{cal}}/\text{kJ/mol}$	$\Delta H^{\text{H}}/\text{kJ/mol}$	$T_{m,1}/^\circ\text{C}$	$\Delta H_1/\text{kJ/mol}$	$T_{m,2}/^\circ\text{C}$	$\Delta H_2/\text{kJ/mol}$
69.6	730	550	66.38 ± 0.02	211.6 ± 2.3	69.99 ± 0.01	516.9 ± 2.1

*Errors in deconvolution parameters are at 95% confidence level. Standard deviation around fit: 1.468

Interestingly, we found that the DSC thermal profile can be well reconstructed by assuming that quinoïn unfolding involves two independent non-two-state transitions occurring at 66 °C and 70 °C, suggesting the presence of two structurally independent domains characterized by a similar thermal stability. An inspection of Fig. 4 suggests that these domains are most likely the N-terminal domain (residues 1–120), which is predominantly β -stranded, and the C-terminal domain, which is mainly α -helical, consistent with the predicted structural features of type 1 RIPs [11].

Notably, these two domains define the cleft where the catalytically active residues are located. Therefore, the ability of the two domains to unfold independently may reflect the required plasticity and flexibility of the active site, further supporting the link between structural dynamics and enhanced activity under acidic conditions.

Concluding remarks

This study provides new insights into how pH and ionic strength influence the structure, thermal stability, and enzymatic activity of quinoïn, a type 1 RIP extracted from *C. quinoa* seeds. Our results demonstrate that quinoïn remains well-folded and thermally stable across a wide pH range, while its PNAG activity is enhanced under acidic conditions and in the presence of physiological salt concentrations. Thermodynamic data suggest that this enhancement may be connected to an increase in the protein structural flexibility and consequently, in the adaptability to its substrate due to the weakening of charge–charge interactions. This finding is particularly relevant, as it indicates that quinoïn can remain functional in the acidic biological environments such as plant vacuoles (pH ~ 5), maintaining its activity despite the pH fluctuations caused by biotic or abiotic stress in the extracellular matrix.

Overall, this study improves our understanding of the structure–function relationship of quinoïn, highlighting its potential biotechnological applications, particularly in plant defence and therapeutic delivery systems.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10973-025-14942-x>.

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Author contributions M.C., I.D.D., R.O., and L.P. carried out the biophysical analysis (CD and DSC analyses); M.C. performed the predictions of protein structure and 3D model analysis; S.R. and N.L. purified the protein and carried out the enzymatic assays; and P.D.V. and A.D.M. conceived the study, wrote the manuscript and acquired the funding. All authors reviewed and edited the manuscript.

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Data availability The raw data supporting the conclusions of this article will be made available by the authors upon request, without undue reservation.

Declarations

Conflict of interest The authors have no conflict of interest to declare.

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