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Purification from *Deinococcus radiodurans* of a 66 kDa ABC transporter acting on peptides containing at least 3 amino acids

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ABSTRACT

Deinococcus radiodurans is a Gram positive bacterium the capability of which to withstand high doses of ionizing radiations is well known. Physiologically speaking, *D. radiodurans* is a proteolytic prokaryote able to express and secrete quite a number of proteases, and to use amino acids as an energy source. When considering this, it is surprising that little information is available on the biochemical components responsible for the uptake of peptides in *D. radiodurans*.
Here we report on the purification and characterization of an ABC peptide transporter, isolated from *D. radiodurans* cells grown in tryptone-glucose-yeast extract (TGY) medium. In particular, we show here that the action of this transporter (denoted DR1571, SwissProt data bank accession number Q9RU24 UF71_DEIRA) is exerted on peptides containing at least 3 amino acids. Further, using tetra-peptides as model systems, we were able to observe that the DR1571 protein does not bind to peptides containing phenylalanine or valine, but associates with high efficiency to tetra-glycine, and with moderate affinity to tetra-peptides containing arginine or aspartate.

Key words: *Deinococcus radiodurans*; ABC transporter; peptides; tryptophan fluorescence.

INTRODUCTION

Deinococcus radiodurans is a Gram positive bacterium featuring an outstanding capability to withstand high doses of ionizing radiations [1,2]. In particular, it was shown that *D. radiodurans* cells can cope with more than a hundred of genomic double-strand breaks generated by the exposure to γ-rays [2,3]. This peculiar DNA repair competence is due to an efficient molecular mechanism denoted as ESDSA, i.e. extensive synthesis-dependent strand annealing [4]. Remarkably, this mechanism takes advantage of the polyploid nature of *D. radiodurans*, the genome multiplicity of which is known to be affected by growth phase and culture medium [5,6]. Beyond this obvious requisite to accomplish the assembly of fragmented chromosomes, the extreme radiation-resistance by *D. radiodurans* was investigated with special emphasis on the maintenance of genomic integrity. Only later it was recognized that the primary reason for radiation-resistance by *D. radiodurans* resides in its ability to successfully face oxidative stress [7]. *Deinococcus radiodurans* does indeed possess biochemical machineries committed to scavenge reactive oxygen species, therefore protecting proteins and enzymes from oxidative damage [8]. Among these biochemical processes, the relevance of manganese in protecting *D. radiodurans* from oxidative stress was investigated in detail [9,10]. These investigations led to the identification of the catalytic competence of complexes of Mn^{2+} with orthophosphate, amino acids, peptides, or nucleosides in scavenging reactive oxygen species, i.e. hydroxyl and superoxide radicals, and hydrogen peroxide [10-13]. In addition, manganese is known to affect the biomass yield of *D. radiodurans* cultures, both in minimal and in rich media [14-16]. Physiologically speaking, *D. radiodurans* is a proteolytic bacterium featuring an energetic metabolism primarily relying on amino acids, which are preferred to carbohydrates as energy sources [17]. When considering this, it is not surprising that *D. radiodurans* was isolated from canned meat subjected to γ-rays [18]. *D. radiodurans* is indeed able to express quite a number of proteases [19-21], along with a repertoire of peptide transporters [19] significantly larger than that of the radiation-sensitive bacterium *Shewanella oneidensis* [22]. The importance of these proteases and peptide transporters extends beyond physiological conditions. Indeed, *D. radiodurans* responds to ionizing radiations by expressing proteases in order to degrade damaged proteins and enzymes, and to possibly recycle amino acids and peptides to restore an intact proteome. In addition to proteases, the expression of *D. radiodurans* peptide transporters is also known to be triggered by exposure of cells to γ-rays [23]. Among these transporters, 4 ABC peptide transporters were described as PHX, i.e. Predicted to be Highly Expressed [20]. However, and surprisingly enough, no detailed biochemical characterization of these transporters is available. Here we report on the *D. radiodurans* DR1571 protein, which is classified as an ABC peptide-binding protein. In particular, the isolation and purification of this protein from *D. radiodurans* cells cultured in TGY medium (and not exposed to ionizing radiations) is shown here, along with the characterization of its binding properties towards a series of peptides of different lengths and amino acid composition.

MATERIALS AND METHODS

Bacterial cultures and media

Deinococcus radiodurans DSM 46620 was obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany), and grown in TGY medium (tryptone, glucose and yeast extract at 5, 1 and 3 g/L, respectively) at 30 °C under constant shaking (200 r.p.m.).

Protein purification

Cell pellets were resuspended in lysozyme solution (10 mg/mL in H_20). After incubation for 1 h at room temperature (under mild shaking), an appropriate volume of buffer was added to obtain 50 mM Tris-HCl, 50 mM NaCl, 1 mM EDTA, 2.5 mM DTT, pH 8. The suspension was supplemented with 1 mM PMSF, cells were disrupted by 3 sonication cycles (18 W, 15 s pulses with 15 s intervals, total time 4 min) followed by 2 cycles of pressure treatment (Aminco French press, 20,000 psi). The crude protein extract accordingly obtained was immediately centrifuged (10,000 x g, 30 min, 4 °C), and the pellet was discarded. Ammonium sulfate was then slowly added to the supernatant to reach 65 % saturation, and the solution was centrifuged at 10,000 x g (30 min, 4 °C). After discarding the pellet, ammonium sulfate was added to the supernatant to obtain 80 % saturation, and the sample was centrifuged. The pellet was resuspended in buffer A (50 mM Tris-HCl, 50 mM NaCl, 1 mM EDTA, pH 8), and dialyzed with the same buffer. The dialyzed solution was loaded onto a HiTrap Heparin column (5 mL) previously equilibrated with buffer A. The column was washed with ten volumes of buffer A, and subsequently a NaCl gradient (50-600 mM) was applied. Fractions (1 mL) were collected and analysed by SDS-PAGE. The best fractions were pooled, concentrated (using an Amicon ultrafiltration cell equipped with a YM30 membrane), and loaded onto a Superdex 200 gel filtration column (16x70) equilibrated with 50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, pH 8. Calibration of the column was performed with HMW and LMW molecular weight standards (GE Healthcare, Piscataway, USA), obtaining the equation $y = 2.166 – 0.354x$, where y is K_{AV} and x is the logarithm of molecular weight. Elution from the column was performed at 0.6 mL/min, and fractions (0.9 mL) were analysed by SDS-PAGE. The best fractions were pooled, concentrated and stored at -20 °C until used. Protein concentration was determined according to Bradford [24].

Mass spectrometry

Separation of peptides were performed as previously described [25]. The resulting peptides were analyzed by LC-MS/MS using an Orbitrap XL instrument (Thermo Fisher Scientific) equipped with a nano-ESI source coupled with a nano-Acquity capillary UPLC (Waters, Milford, USA). Briefly, peptides were separated with a capillary BEH C18 column using aqueous 0.1 % formic acid (A) and CH_3CN containing 0.1 % formic acid (B) as mobile phases. Peptides were eluted by means of a linear gradient from 5 to 50 % of B in 90 minutes, at a 300 nL/minute flow rate. Mass spectra were acquired over an *m/z* range from 400 to 1800. To achieve protein identification, MS and MS/MS data underwent Mascot Search Engine software analysis to interrogate the National Center for Biotechnology Information non redundant (NCBInr) protein database. Parameters sets were: trypsin cleavage; carbamidomethylation of cysteines as a fixed modification, and methionine oxidation as a variable modification; a maximum of two missed cleavages; false discovery rate, calculated by searching the decoy database, was set at 0.05.

Synthesis of peptides

The peptides were assembled by SPPS on a commercially available Wang resin preloaded with Fmoc-Gly, using Fmoc N-protected amino acids; Arg and Asp were introduced as Fmoc-Arg(Pbf)-OH and Fmoc-Asp(OtBu)-OH, respectively. The Fmoc-Gly preloaded resin (0.15 g, Gly loading 0.4-0.8 mmol/g) was treated with 25 % piperidine in DMF (4mL) and shaken for 20 minutes. The suspension was filtered and the resin was washed in sequence with 5 mL each of CH_2Cl_2, MeOH, and DMF. The

resin was then treated with 25 % piperidine in DMF and shaken for 40 minutes. The suspension was filtered and the resin was washed twice with 5 mL each of CH_2Cl_2, MeOH, DMF and Et_2O. The Fmoc-amino acid (0.23 mmol), was activated with HOBt (0.23 mmol), in 2mL of DMF for 10 min and added to the H-Gly-resin. Then TBTU (0.23 mmol) and DIPEA (0.36 mmol) were added and the suspension was mixed for 3 h. The resin was filtered and washed twice with 5 mL each of CH_2Cl_2, MeOH, DMF and Et_2O. Coupling efficacy was determined by means of the Kaiser test. Finally, the peptidyl resin was treated with a mixture of TFA and TIPS/water/PhOH as scavengers (88:5:5:2 v/v, 10 mL), for 2 h at room temperature. The mixture was filtered, and the resin was washed 3 times with 5% TFA in CH_2Cl_2 (5 mL), 5% TFA in Et_2O (5mL) and Et_2O (10 mL) in sequence. The filtrate and the washes were collected and solvent and volatiles were removed by nitrogen flow at room temperature. The resulting residue was suspended in ice-cold Et_2O (15 mL) and the crude solid was triturated, collected by centrifugation, resuspended in Et_2O and centrifuged again. Purities were determined to be > 95 % by analytical RP HPLC and ESI mass spectrometry (Supplementary Figures S1-S4).

Binding assays

The binding of peptides to the *Deinococcus radiodurans* DR1571 transporter was assayed in 96 microwells plates by detecting the fluorescence of protein tryptophanes. Reaction mixtures containing the peptide transporter (400 nM) and the ligand to be assayed were prepared in 50 mM Tris-HCl, 50 mM NaCl, pH 8, in a final volume of 100 μL. The fluorescence of the samples was determined with a Perkin-Elmer Enspire microplate reader, using 280 and 340 nm as the excitation and emission wavelengths, respectively.

The kinetics of tetraglycine binding to the DR1571 transporter was assayed using a KinTek SF2004 stopped-flow instrument (KinTek, Snow Shoe, PA, USA). The protein syringe was filled with 1 μM peptide transporter in 50 mM Tris-HCl, 50 mM NaCl, pH 8. The peptide syringe was filled with 200 μM tetraglycine in the same buffer. The change in protein tryptophanes fluorescence was observed at 20 °C, exciting samples at 280 nm and monitoring the emission using a longpass filter. For each measurement, 20 traces were averaged.

RESULTS AND DISCUSSION

We have recently compared the proteomes of *Deinococcus radiodurans* cells grown under physiological conditions in TGY medium supplemented or not with Mn^{2+} [16]. Surprisingly enough, we did observe that the enrichment of the medium with manganese significantly increases the pI and down-regulates the expression of the iron and phosphate ABC transporters, respectively [16]. Due to its proteolytic nature, *D. radiodurans* expresses 4 ABC peptide transporters [20], whose level was not affected by the addition of manganese to TGY medium [16]. Accordingly, the *D. radiodurans* peptide ABC transporters represent essential physiological components of this microorganism, to which they are most likely indispensable to recover from oxidative stress. We therefore thought it of interest to attempt the purification of a peptide transporter from *Deinococcus radiodurans* cells grown in TGY medium. To this aim, we used the biomass obtained from 1 L of culture, and we extracted the soluble protein fraction from this sample. As a first purification step, we performed a precipitation with ammonium sulfate (at 65 % saturation), which was found to be very useful to discard the majority of the lysozyme used to increase the yield of proteins extraction. Further ammonium sulfate (to reach 80 % saturation) was added to the protein soluble fraction obtained after the previous step, and after dialysis the sample was loaded onto a HiTrap Heparin column. After an extensive washing of the column with the equilibration buffer (see Methods), a gradient of NaCl was applied, letting to isolate two major *D. radiodurans* proteins: i) glycerol 3-phosphate dehydrogenase, which was eluted at approximately 0.35 M NaCl; ii) and "Probable ABC transporter-binding protein DR_1571" (SwissProt data bank, Q9RU24 UF71_DEIRA), eluted at about 0.45 M NaCl concentration (Fig. 1A). These two proteins were identified by mass spectroscopy after an SDS-PAGE analysis of the fractions corresponding to the 2 major peaks of the chromatogram (Fig. 1 A, Tables 1 and 2, Supplementary Figure S5). The most similar protein to DR1571 was found to be the peptide/nickel transport system substrate-binding protein from *Deinococcus hopiensis* KR-140, which shares 76 % identity with the *D. radiodurans* protein. The best fractions eluted from the affinity chromatography column and containing DR1571 were pooled, concentrated and loaded onto a Superdex 200 gel filtration column. This chromatographic step was useful to remove low-molecular weight contaminants (Fig.s 1B and 1C-1D). Taking into account the calibration of the Superdex 200 column (see Methods), we estimated the molecular mass of DR1571 as equal to 59.7 kDa, in good agreement with the expected value of 65.6 kDa for this protein in monomeric form. Peptide-binding proteins are located in the periplasm of Gram negative bacteria [26], and in Gram positives they are associated with the plasmamembrane, to which are anchored by a hydrophobic N-ter region [27]. Therefore, it is important to note that we were able to purify the DR1571 peptide-binding protein in soluble form, i.e. detached from the *D. radiodurans* plasmamembrane, most likely because of the sufficiently harsh extraction procedure we used.

As a first test, we assayed the binding, if any, of glycine and diglycine to DR1571. To this aim, we decided to test the fluorescence of protein tryptophanes in the absence or in the presence of potential ligands. When the fluorescence of tryptophanes was determined in the absence of ligands and as a function of protein concentration, we observed a linear dependence up to 600 nM DR1571 (Supplementary Figure S6). Further, using 400 nM protein as a constant concentration of target we assayed fluorescence as a function of glycine or di-glycine concentration (Fig. 2A). No significant differences were observed when these samples were compared with those represented by free protein (Fig. 2A), suggesting that DR1571 is not able to bind glycine or di-glycine. Alternatively, the binding of glycine or di-glycine would not trigger conformational rearrangements of DR1571 altering tryptophanes fluorescence. However, when tri- or tetra-glycine were assayed, a significant binding did occur, and the corresponding K_D values were estimated (Fig. 2B), yielding 18.7 ± 6.8 and 19.5 ± 5.4 μm for tri- and tetra-glycine, respectively. Finally, when penta-glycine was tested a higher

affinity for this ligand was observed, corresponding to a K_D equal to 5.2 $\pm$ 0.7 μm (Fig. 2C). Overall, these observations suggest that DR1571 does not bind di-peptides, features similar affinities for tri- and tetra-peptides, and higher affinity for penta-peptides. The OppA peptide-binding protein from *Escherichia coli* was characterized using a similar approach to that we used to investigate DR1571, i.e. OppA was assayed for the binding of di-, tri-, tetra- and penta-alanine [28]. Interestingly, no significant binding of di-alanine was detected, tri- and tetra-alanine were bound with high affinity, and the binding of penta-alanine occurred with an affinity two orders of magnitude lower than those determined at the expense of tri- and tetra-alanine [28]. More recently, the absence of binding to di-peptides by *E. coli* OppA was confirmed [29]. In addition, the binding of tri- and tetra-peptides containing different amino acids was quantitatively determined, yielding K_D values in the nM to μM range [29]. Similar observations were obtained for the binding of tri-peptides by the OppA protein from *Salmonella typhimurium*, with observed K_D values up to 6 μM [30]. Interestingly, when compared to *E. coli* and *S. typhimurium* OppA proteins, the orthologue peptide-binding protein from the Gram positive *Bacillus subtilis* features lower affinities, i.e. in the μM range for tri-, tetra- and penta-peptides [31]. In particular, penta-peptides appear to be preferred by *B. subtilis* OppA (K_D = 1.00 ± 0.03 μM), and tri-peptides are bound with the lower affinity (K_D = 50.92 ± 4.30 μM) [31], a behaviour qualitatively similar to that reported here for *D. radiodurans* DR1571 peptide-binding protein.

Further, we assayed the binding to DR1571 of tetra-peptides containing 3 glycines and arginine, phenylalanine, valine, or aspartate at the third position, i.e. GGRG, GGFG, GGVG, and GGDG. Using concentrations of GGFG or GGVG up to 1.2 mM, no significant fluorescence changes of DR1571 tryptophanes were observed (Fig. 3A), suggesting that the ABC transporter does not associate to these tetra-peptides. However, when binding assays were performed with GGRG or GGDG a significant binding was observed, and the affinity of DR1571 for these tetra-peptides was found to be essentially similar (Fig. 3B). Surprisingly, we estimated K_D values for the GGRG-DR1571 and GGDG-DR1571 complex one order of magnitude higher than the value determined for the GGGG-DR1571 complex. Indeed, we calculated K_D values equal to 385 ± 99 and 443 ± 136 μM for the GGRG-DR1571 and the GGDG-DR1571 complex, respectively (Fig. 3B).

Finally, we wanted to test the kinetics of the association of DR1571 to tetra-glycine. This binding was assayed using a stopped-flow instrument over two time intervals, i.e. 10 and 60 seconds, and monitoring the fluorescence of DR1571 tryptophanes. As shown if Figure 4A, a significant decrease of tryptophanes fluorescence was detected during the first seconds of the binding reaction (assayed in the presence of 500 nM DR1571 and 100 μM tetra-glycine). Interpreting this kinetics with a double-exponential equation, we obtained k_{obs} values equal to 1.0 ± 0.1 and 0.072 ± 0.008 s^{-1}, for the fast and slow phase, respectively (the corresponding amplitudes were estimated equal to 32 ± 3 and 282 ± 15 mV). When a longer time interval was used for the assays, the binding of tetra-glycine was observed at almost completion (Fig. 4B), yielding 0.47 ± 0.06 and 0.034 ± 0.006 s^{-1} for the fast and slow phase, with amplitudes equal to 49 ± 3 and 392 ± 11 mV, respectively. Further, we assayed the kinetics of GGRG and GGDG binding to the DR1571 transporter, under the same conditions used to test the binding of tetra-glycine. Remarkably, the values of the rate constants observed for the binding of GGRG and GGDG to DR1571 were determined to be very similar to those obtained for tetra-glycine (Supplementary Figs. S7-S8, Supplementary Table 1). Overall, these values are much lower when compared with those determined for the binding of *Lactococcus lactis* OppA protein to different peptides [32]. In particular the binding of *L. lactis* OppA to octa-, nona- or dodeca-peptides was observed to occur with k_{obs} values ranging from 200 to 400 s^{-1} when the ligands were at 20-100 μM in the assay mixtures [32]. Notably, the better performance in kinetics of peptide binding by *L. lactis* OppA when compared to DR1571 is somewhat related to differences in affinities for the ligands. In particular, *L. lactis* OppA features K_D values for nona- and dodeca-peptides equal to 1-2

μM [32].
As a final test, the kinetics of penta-glycine binding to DR1571 was assayed. As expected from the outcome of equilibrium binding experiments (Fig.s 2B-2C), the association of this penta-peptide to the ABC transporter occurred significantly faster when compared to the binding reactions observed in the presence of tetra-peptides. Indeed, by assaying over a 10 s time interval the association of penta-glycine to DR1571, and interpreting the observed kinetics with a double-exponential equation, we obtained k_{obs} values equal to 3.19 ± 0.07 and 0.115 ± 0.009 s^{1} for the fast and slow phase of the reaction, respectively (Fig. 4C). It should be noted that the amplitude of the DR1571 fluorescence decrease triggered by the binding of penta-glycine was not significantly higher than the amplitudes determined for the binding of tetra-peptides (Supplementary Table 1), suggesting that similar conformational transitions are induced by the different peptides tested. We also assayed the binding of penta-glycine over a 60 s time interval. However, due to the relatively fast binding of this penta-peptide to DR1571, the initial phase of the reaction was not appropriately resolved, yielding high residuals (Fig. 4D).
We have shown here that the D. radiodurans DR1571 protein is a transporter of peptides containing at least 3 amino acids, with penta-peptides bound with higher affinity than tetra-peptides. To investigate the relevance, if any, of DR1571 in D. radiodurans oxidative stress resistance, further work will be performed to assay the binding of $Mn^{2þ}$-peptide complexes by DR1571.

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Appendix A. Supplementary data

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FIGURE LEGENDS

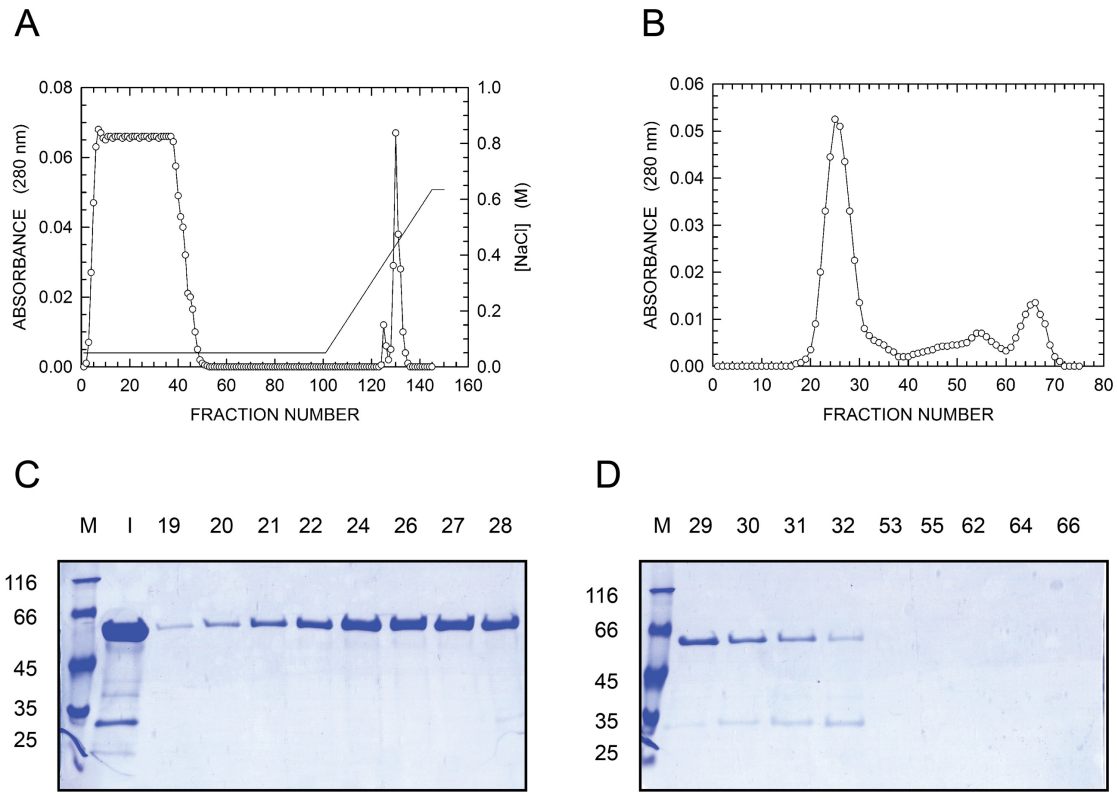


Figure 1

Purification of the *Deinococcus radiodurans* DR1571 peptide-binding protein. **A)** Affinity chromatography (HiTrap Heparin column, 5 mL) of the protein fraction obtained by ammonium sulfate precipitation (65-80 % saturation). SDS-PAGE analysis revealed the presence of glyceraldehyde 3-phosphate dehydrogenase and of DR1571 in the first and the second peak of the chromatogram, respectively (Table 1, Supplementary Fig. S1). **B)** Gel filtration chromatography (Superdex 200 column) of fractions 129-131 isolated from the previous step (Supplementary Fig. S1). **C,D)** SDS-PAGE of some representative fractions eluted from the gel filtration column. The molecular masses of the standards used are indicated at the left side. M: molecular mass markers. I: input.

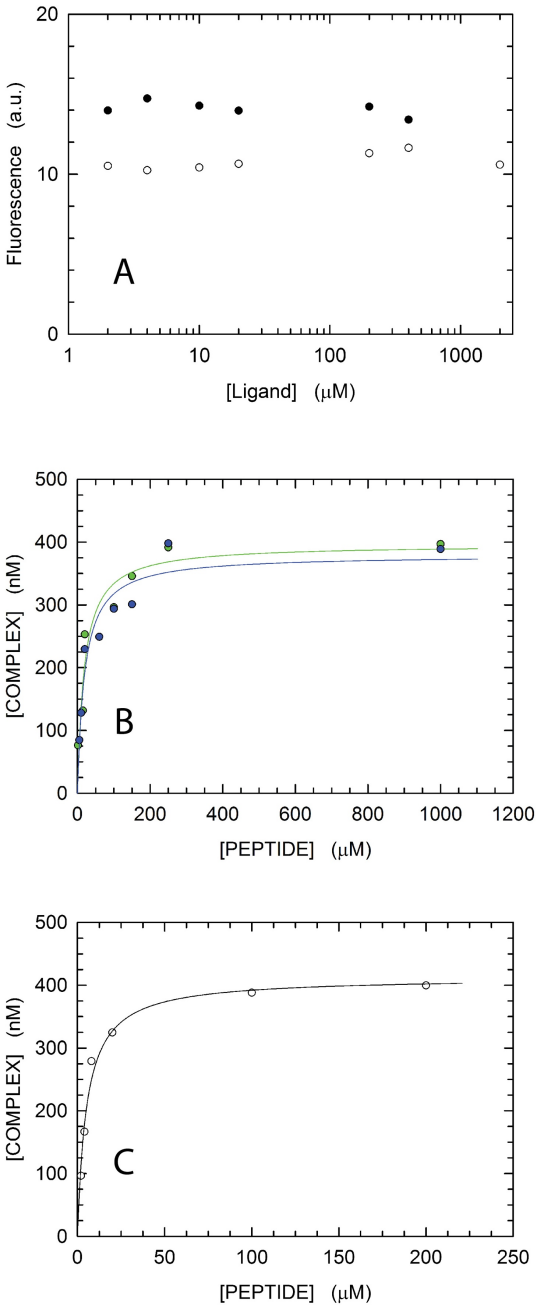


Figure 2

Binding of peptides by *Deinococcus radiodurans* DR1571 protein. **A)** Fluorescence of 400 nM DR1571 in the presence of variable concentrations of glycine (empty circles) or di-glycine (filled circles), in 50 mM Tris-HCl, pH 8. The fluorescence of the target protein in the absence of any ligand was determined as equal to 12.94 ±0.43 (arbitrary units). **B)** Variable concentrations of tri-glycine (green circles) or tetra-glycine (blue circles) were mixed with 400 nM DR1571, in 50 mM Tris-HCl, pH 8. At equilibrium, the concentration of protein-peptide complex was determined by assaying the fluorescence of DR1571 tryptophanes. The continuous lines represent the best fits of a rectangular hyperbola equation to the experimental observations. **C)** Variable concentrations of penta-glycine were mixed with 400 nM DR1571, and the concentrations of protein-peptide complex were determined as in Fig. 2B. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

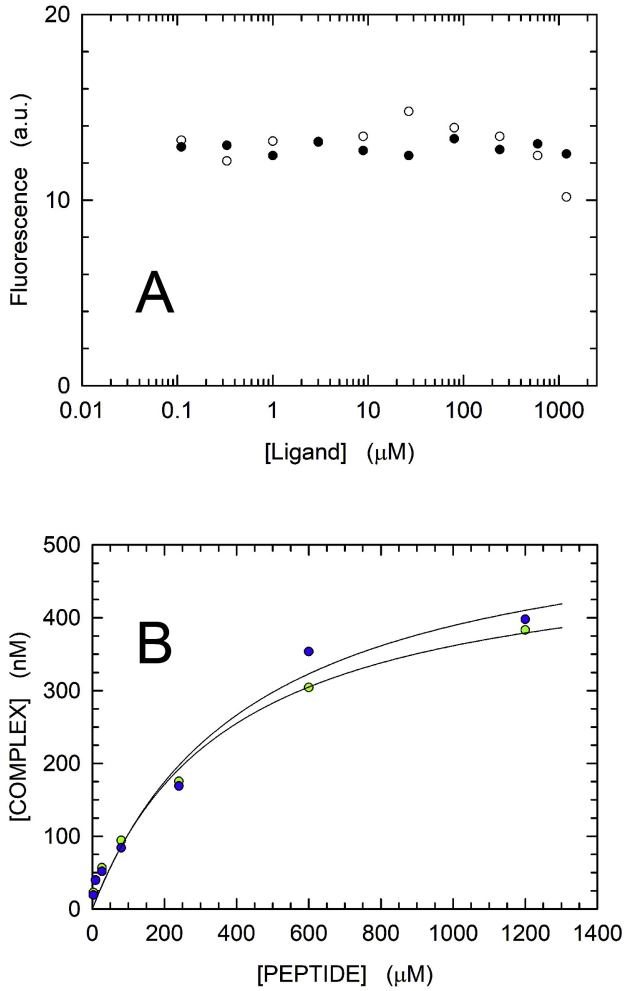


Figure 3

Binding of peptides by *Deinococcus radiodurans* DR1571 protein. **A)** Fluorescence of 400 nM DR1571 in the presence of variable concentrations of GGFG (empty circles) or GGVG (filled circles), in 50 mM Tris-HCl, pH 8. The fluorescence of the target protein in the absence of any ligand was determined as equal to 13.47 $\pm$ 0.15 (arbitrary units). **B)** Variable concentrations of GGRG (green circles) or GGDG (blue circles) were mixed with 400 nM DR1571, in 50 mM Tris-HCl, pH 8. At equilibrium, the concentration of protein-peptide complex was determined by assaying the fluorescence of DR1571 tryptophanes. The continuous lines represent the best fits of a rectangular hyperbola equation to the experimental observations.

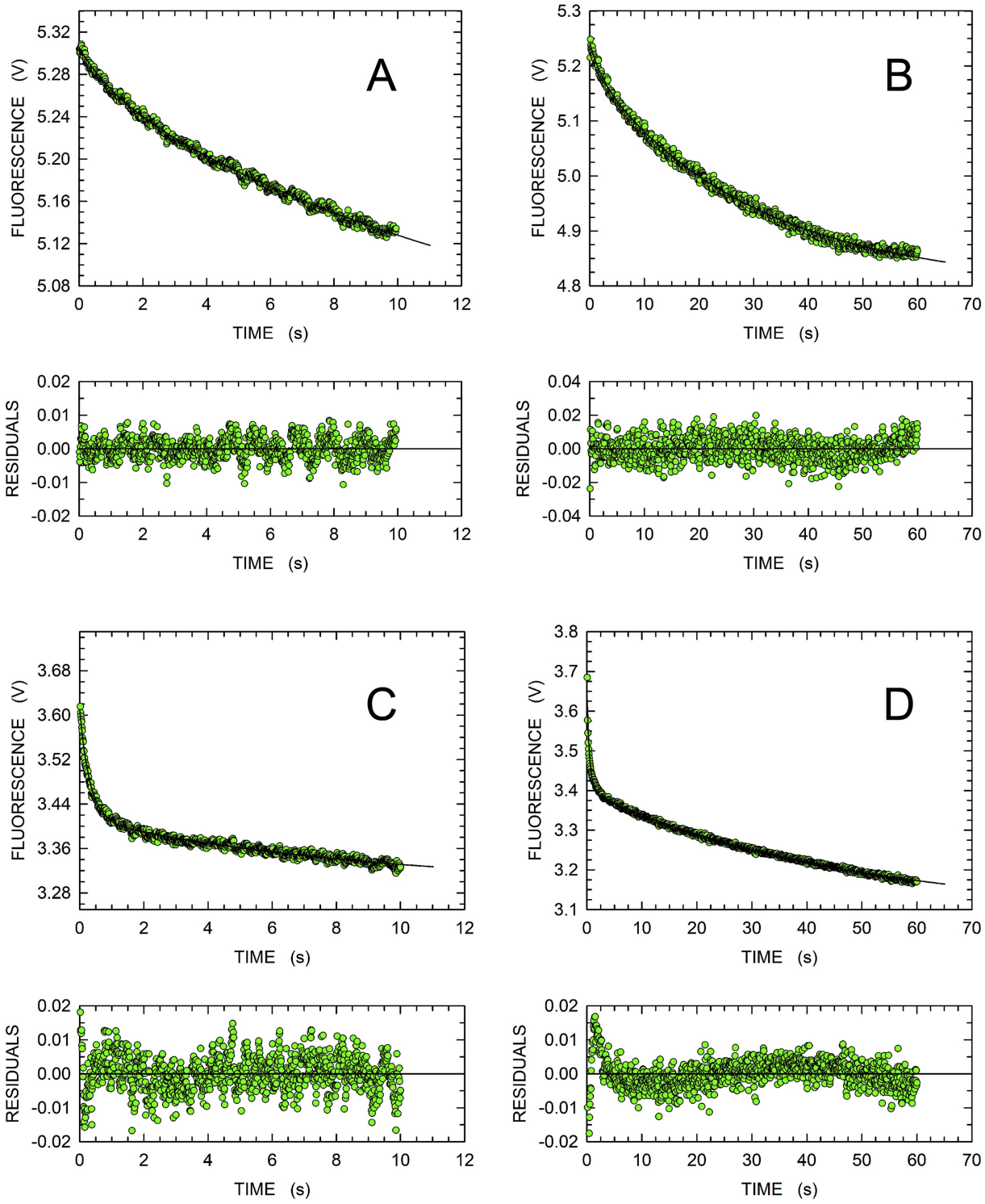


Figure 4

Kinetics of tetra-glycine binding by *Deinococcus radiodurans* DR1571 protein. **A,B)** The binding of tetra-glycine was assayed by a stopped-flow instrument. The protein syringe contained 1 μM DR1571 in 50 mM Tris-HCl, pH 8. The tetra-glycine syringe was filled with 200 μM peptide in the same buffer used for the target protein. The continuous lines represent the best fit of a double-exponential equation to the experimental observations. **C,D)** Stopped-flow assays of penta-glycine binding. The protein syringe contained 1 mM DR1571 in 50 mM Tris-HCl, pH 8. The penta-glycine syringe was filled with 200 mM peptide in the same buffer used for the target protein. Other conditions as those used for the assays of tetra-glycine binding.

Table 1

Identification of Deinococcus radiodurans probable ABC transporter (Q9RU24 UF71_DEIRA) by mass spectrometry of peptides obtained by in-gel tryptic digestion.

Peptide	Observed M_r (Da)	Theoretical M_r (Da)	Difference (ppm)
43-50	949,487	949,488	1,1
109-130	2524,212	2524,218	2,4
131-148	2110,996	2111,001	2,4
164-189	2942,540	2942,548	2,7
198-219	2509,162	2509,168	2,4
231-239	1153,519	1153,523	3,5
307-323	1911,802	1911,809	3,7
343-366	2558,261	2558,267	2,3
409-421	1533,779	1533,785	3,9
440-457	1949,029	1949,033	2,1
509-521	1625,824	1625,830	3,7
564-585	2460,251	2460,257	2,4
586-594	1114,650	1114,651	0,9

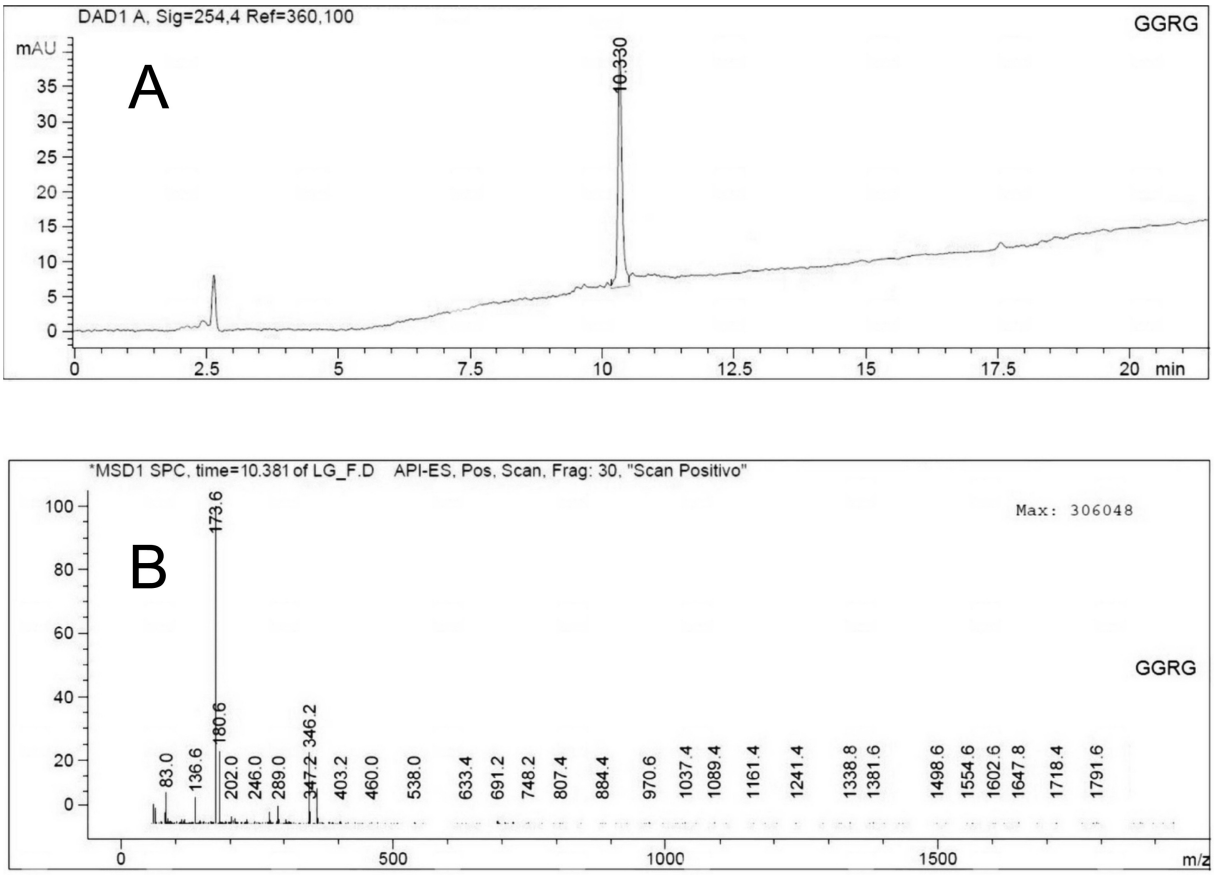
Table 2

Identification of Deinococcus radiodurans Glycerol-3-phosphate dehydrogenase (Q9RR76_GPDA_DEIRA) by mass spectrometry of peptides obtained by in-gel tryptic digestion.

Peptide	Observed M_r (Da)	Theoretical M_r (Da)	Difference (ppm)
36-42	831,435	831,436	1,3
97-105	930,521	930,523	2,6
114-125	1297,630	1297,636	3,1
126-140	1547,805	1547,812	4,2
152-169	1810,961	1810,965	2,1
175-186	1181,665	1181,666	0,8
187-206	1921,968	1921,980	5,2
223-250	2830,398	2830,403	0,9
261-269	886,414	886,414	0
276-295	2083,096	2083,102	2,8
303-312	1086,509	1086,513	3,2

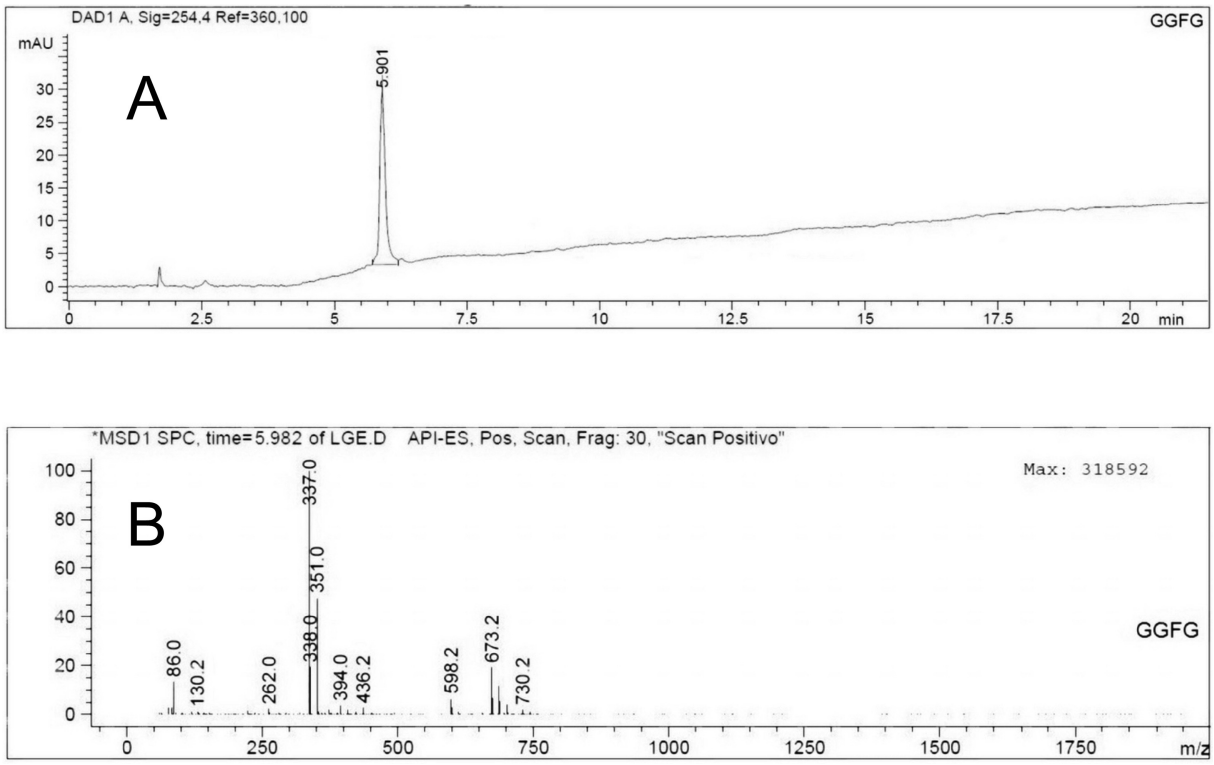
Supplementary Table 1. Kinetic parameters determined for the association reaction of tetra-peptides (GGGG, GGRG, and GGDG) or penta-glycine to *Deinococcus radiodurans* DR1571 peptide-binding protein.

PEPTIDE	k_{obs} 1 (s^{-1})	Amplitude 1 (mV)	k_{obs} 2 (s^{-1})	Amplitude 2 (mV)
GGGG (10 s)	0.95 ± 0.11	32 ±3	0.072 ± 0.008	282 ± 15
GGGG (60 s)	0.47 ± 0.06	49 ± 3	0.034 ± 0.006	392 ± 11
GGRG (10 s)	1.95 ± 0.28	12 ± 1	0.063 ± 0.003	242 ± 7
GGRG (60 s)	0.17 ± 0.01	64 ± 3	0.011 ± 0.001	710 ± 17
GGDG (10 s)	0.89 ± 0.19	14 ± 2	0.061 ± 0.007	264 ± 18
GGDG (60 s)	0.23 ± 0.02	49 ± 2	0.014± 0.001	581 ± 10
GGGGG (10 s)	3.19 ± 0.07	196 ± 2	0.115 ± 0.009	122 ± 4



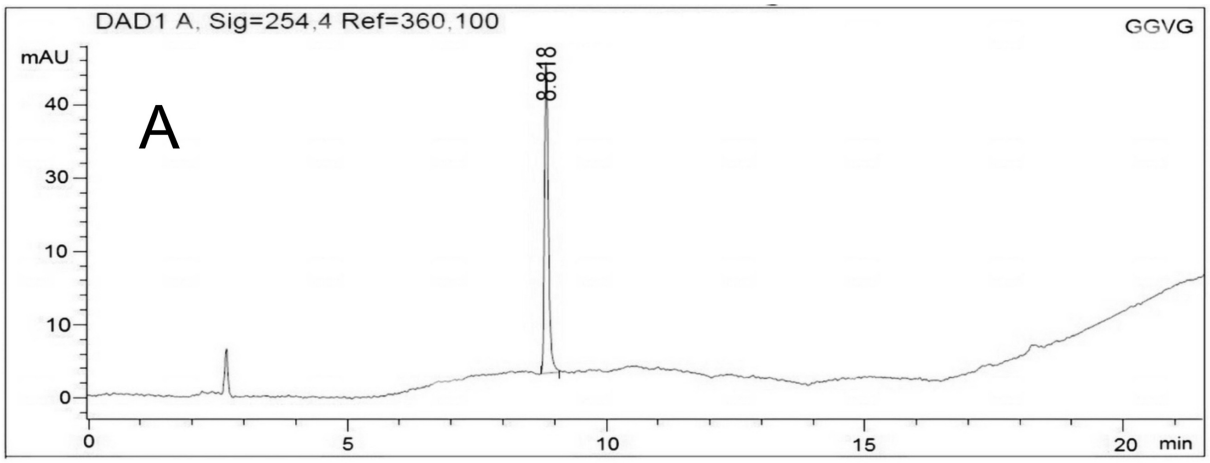
Supplementary Figure S1

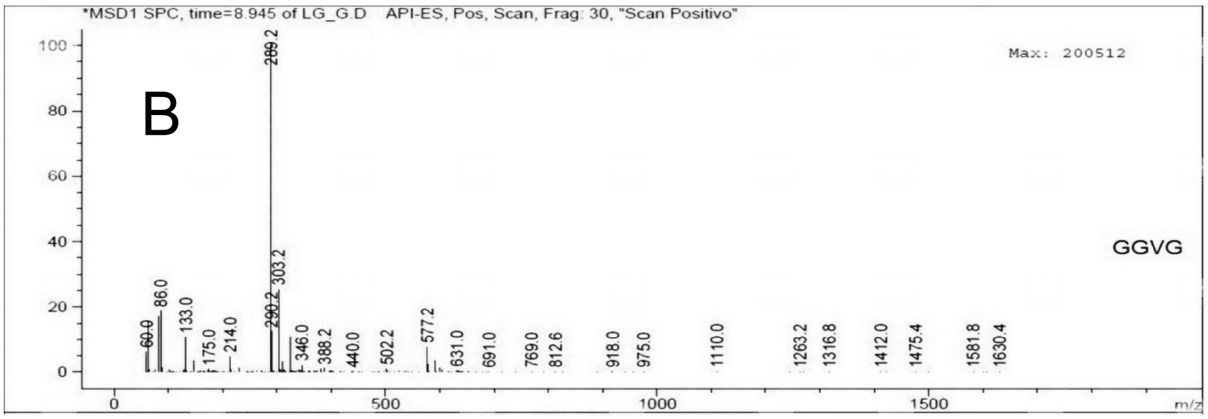
Analysis of H-Gly-Gly-Arg-Gly-OH peptide. **A,B)** The RP HPLC chromatogram (A) features a single peak eluted at 10.330 minutes, and the ESI mass spectrum (B) contains 3 expected peaks at m/z equal to 173.6 (m+2), 346.2 (m+1), and 691.2 (2m+1). RP HPLC was performed with an Agilent 110 Series apparatus, using a RP column Waters Xselect Peptide CSH^{TM}C18 (130 Å, 3.5 µm, 4.6x100 mm) and a diode array detector (254 nm). Solvent A was 0.1% TFA in 95:5 H_2O/CH_3CN, and solvent B was 0.07% TFA in 5:95H_2O/CH_3CN. The elution program consisted of 95:5 A/B for 1 min, then from 95:5 to 5:95 A/B in 15 min, followed by 10 min at the same composition, flow rate equal to 0.7 mL/min. ESI mass spectrometry was carried out with a MS single quadrupole HP 1100MSD detector, with a drying gas flow of 12.5 L/ min, nebulizer pressure 30 psi, drying gas temp 350 °C, capillary voltage 4500 (1) and 4000 (2), scan 50 -2600 amu.



Supplementary Figure S2

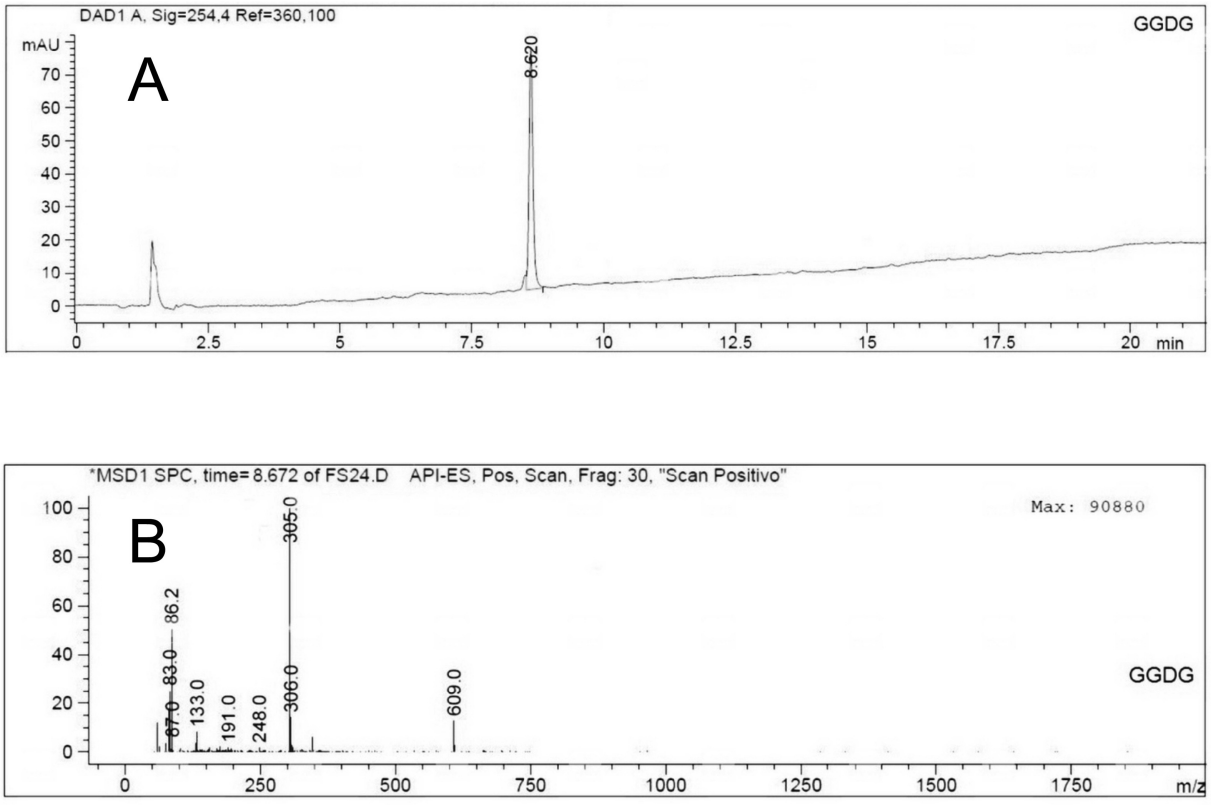
Analysis of H-Gly-Gly-Phe-Gly-OH peptide. **A,B)** The RP HPLC chromatogram (A) features a single peak eluted at 5.901 minutes, and the ESI mass spectrum (B) contains 2 expected peaks at m/z equal to 337.0 (m+1), and 673.2 (2m+1). Analytical conditions were the same as those described for Supplementary Figure S1.





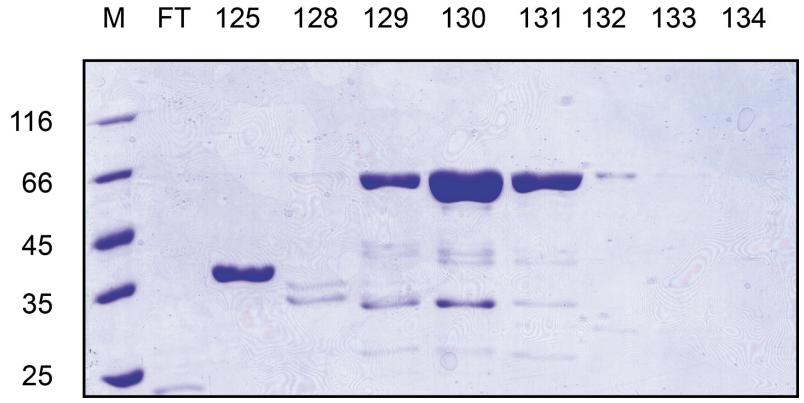
Supplementary Figure S3

Analysis of H-Gly-Gly-Val-Gly-OH peptide. **A,B)** The RP HPLC chromatogram (A) features a single peak eluted at 8.818 minutes, and the ESI mass spectrum (B) contains 2 expected peaks at m/z equal to 289.2 (m+1), and 577.2 (2m+1). Analytical conditions were the same as those described for Supplementary Figure S1.



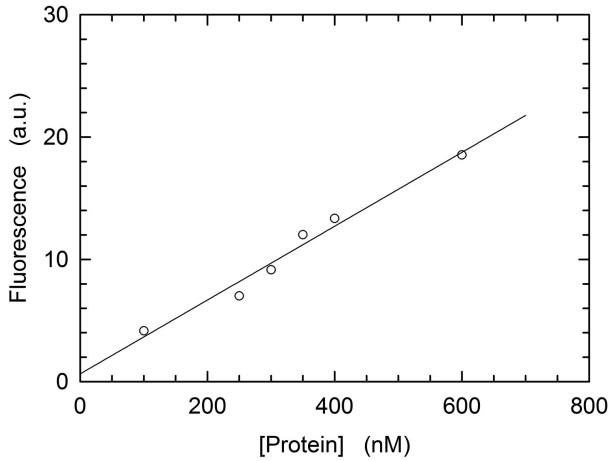
Supplementary Figure S4

Analysis of H-Gly-Gly-Asp-Gly-OH peptide. **A,B)** The RP HPLC chromatogram (A) features a single peak eluted at 8.620 minutes, and the ESI mass spectrum (B) contains 2 expected peaks at m/z equal to 305.0 (m+1), and 609.0 (2m+1). Analytical conditions were the same as those described for Supplementary Figure S1.



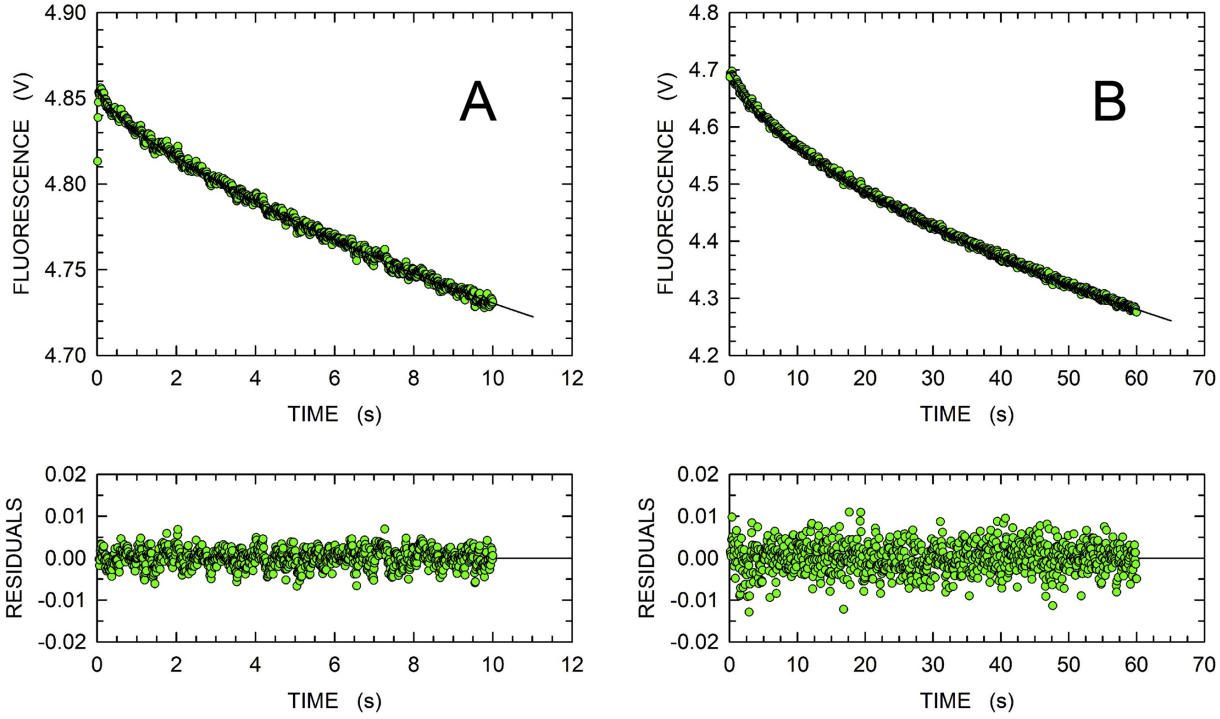
Supplementary Figure S5

SDS-PAGE of some representative fractions eluted from the Hi-Trap Heparin column used to purify the *Deinococcus radiodurans* DR1571 peptide-binding protein. The molecular masses of the standards used are indicated at the left side. M: molecular mass markers. FT: flow-through.



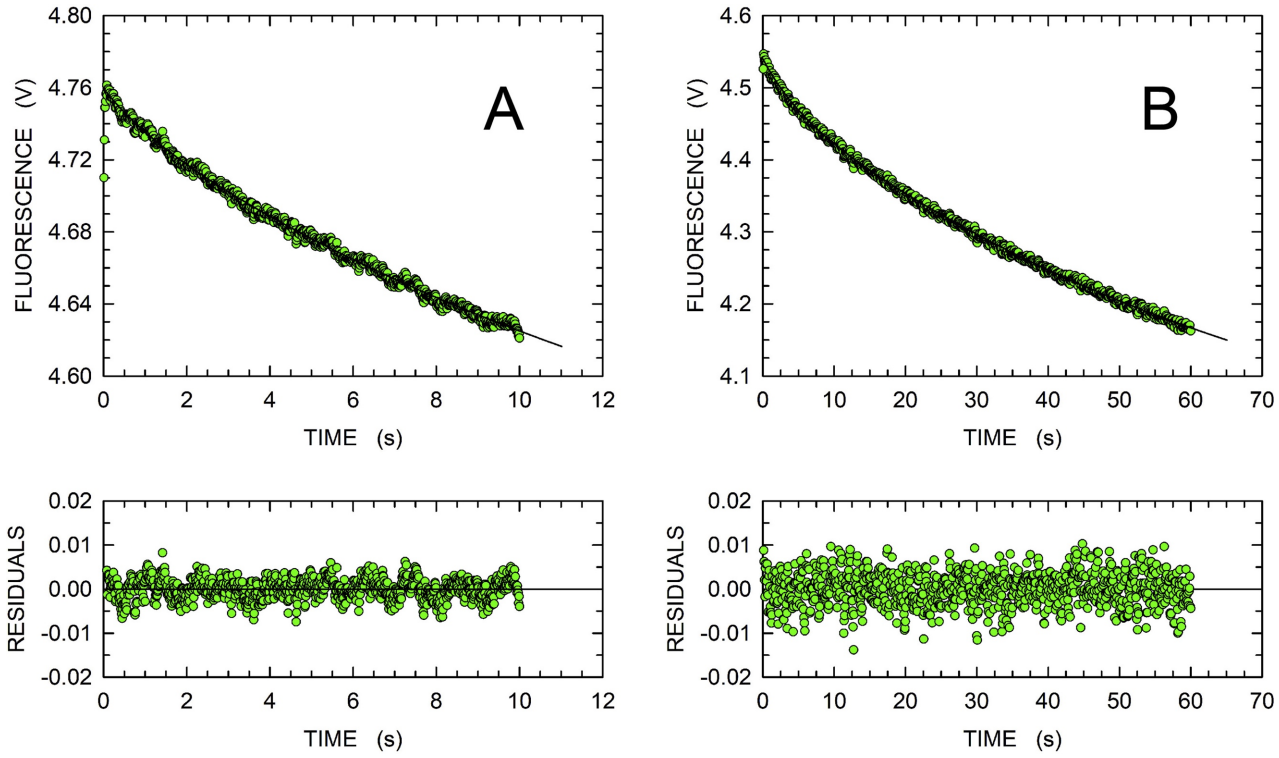
Supplementary Figure S6

Fluorescence of *Deinococcus radiodurans* DR1571 as a function of protein concentration, in the absence of any ligand. The assay mixtures contained variable concentrations of DR1571 in 50 mM Tris-HCl, pH 8. The different samples were excited at 280 nM, and the emission by protein tryptophanes was detected at 340 nm.



Supplementary Fig. S7.

Kinetics of GGRG binding by *Deinococcus radiodurans* DR1571 protein. **A,B)** The binding of GGRG was assayed by a stopped-flow instrument using 280 nm as the exciting wavelength, and detecting DR1571 tryptophanes fluorescence with a longpass filter. The protein syringe contained 1 µM DR1571 in 50 mM Tris-HCl, pH 8. The GGRG syringe was filled with 200 µM peptide in the same buffer used for the target protein. After rapid mixing, fluorescence changes were recorder over a 10 (A) or 60 (B) seconds time interval. Assay temperature was 20 °C. The continuous lines represent the best fit of a double-exponential equation to the experimental observations. The best fit in (A) was obtained excluding the initial fast fluorescence increase.



Supplementary Fig. S8.

Kinetics of GGDG binding by *Deinococcus radiodurans* DR1571 protein. **A,B)** The binding of GGDG was assayed by a stopped-flow instrument using 280 nm as the exciting wavelength, and detecting DR1571 tryptophanes fluorescence with a longpass filter. The protein syringe contained 1 µM DR1571 in 50 mM Tris-HCl, pH 8. The GGDG syringe was filled with 200 µM peptide in the same buffer used for the target protein. After rapid mixing, fluorescence changes were recorder over a 10 (A) or 60 (B) seconds time interval. Assay temperature was 20 °C. The continuous lines represent the best fit of a double-exponential equation to the experimental observations. The best fit in (A) was obtained excluding the initial fast fluorescence increase.