Characterization of a lunasin-derived anti-inflammatory peptide

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Lunasin: anti-inflammatory peptide

Lunasin is a bioactive soy peptide containing 43 amino acids which is encoded within a 2S-albumin protein. Several studies have shown lunasin has potential in the development of treatments for cancer, anti-inflammatory diseases and cardiovascular diseases. Despite its small size, lunasin contain several motifs as shown in Figure 1A and the structure appears to be intrinsically disordered with transiently populated helical regions as shown in Figure 1B. However, both the N- and C-termini are predicted to be disordered (Figure 2).

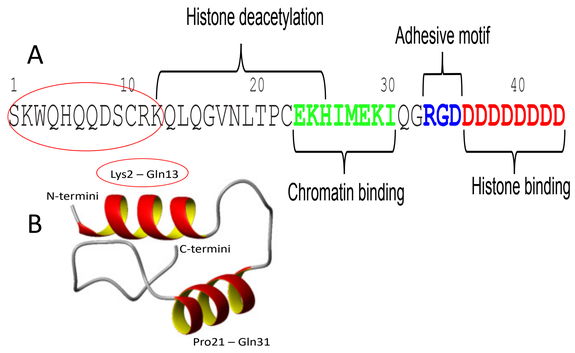

Figure 1: (A) Sequence of lunasin with active domains responsible for its bioactivities.**(B)** The 3D-modelled structure (PEP FOLD 3) of lunasin comprising two helical regions. The segment at the N-termini, circled in red, is the subject of the current study.

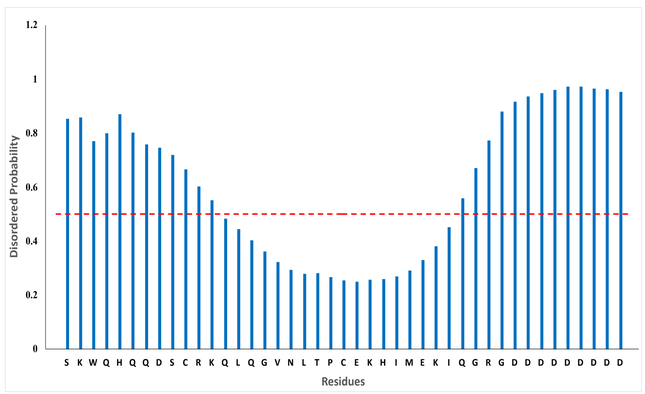

Figure 2: Lunasin Disordered probability. Red dotted line is the probability threshold at 0.5. Residues with >0.5 are considered disordered and residues with <0.5 are considered ordered. Residues 1-12 and 31-43 are disordered whereas residues 13-30 are predicted to be ordered.

Sequence Homology

Regions of lunasin have sequence homology with a range of different proteins and motifs. In addition to the region with homology to chromatin binding proteins and the RGD motif, the N-terminal region, residues 3-12, has homology with uncharacterised proteins from bacteria (Figure 3). Interestingly, gene coding has suggested that lunasin has a microbial origin which might indicate a link with these proteins.

Source	Protein	Sequence	% Identity	Accession code
Soybean (Glycine Max)	2S-albumin precursor	WQHQQDSCRK	100	NP_001238443
Glycine Soja	2S-albumin	WQHQQDSCRK	100	XP_028186255
Bacteria (*Microbiospora rosea*)	hypothetical protein	WQHLQDSCRK	89	WP_076440832
Bacteria (*Microbiospora hainanensis*)	hypothetical protein	WQHLQDSCRK	89	WP_142620042
Bacteria (*Microbispora sp. H13382*)	hypothetical protein	WQHLQDSCRK	89	WP_182907491
Bacteria (*Microbispora sp. H10837*)	hypothetical protein	WQHVQDSCRK	89	WP_169986856

Figure 3: Sequence Homology of the N-terminal region of lunasin. Sequence Blast using NCBI protein database deduced 89 % sequence homology with hypothetical disordered protein from bacteria (*Microbispora sp)*.

Structural analysis

A peptide corresponding to residues 3-12 of lunasin (LN10) was synthesised using solid phase peptide synthesis. The structure was analysed using NMR spectroscopy (Figure 4) and the effect on TNFα suppression analysed (Figure 5).

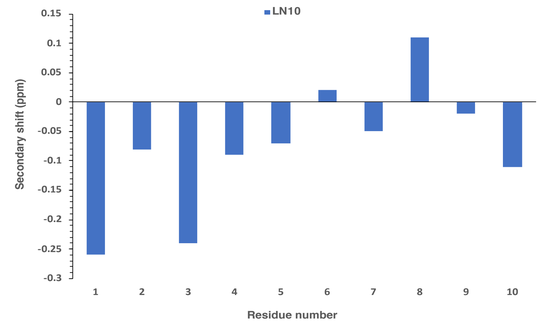

Figure 4: The secondary shifts of LN10. The shifts were generated by subtracting the αH shifts from random coil shifts. The majority of the shifts are within +/-0.1 ppm indicative of an unstructured peptide.

Hydrogen/Deuterium exchange

LN10 was dissolved in 100% D_2O and NMR spectra recorded over time (24 hours) to allow measurement of amide deuterium exchange rates. Amide exchange rates (kex) were classified in the following ranges: Fast (kex>4.16min^{-1}), Intermediate (4.16>kex>0.1 min^{-1}) and Slow (0.1>kex>0.002 min^{-1}). The majority of the residues were in fast exchange, but a peak corresponding to either Arg9/Lys10 was in intermediate exchange. This lack of slowly exchanging amide protons is consistent with the peptide being unstructured.

Cytokine analysis

Cytokine analysis of LN10 was analysed in both fresh and frozen PBMCs (Figure 5). The reduction of the multifunctional cytokine (TNFα) by LN10 is indicative of the peptide having anti-inflammatory properties.

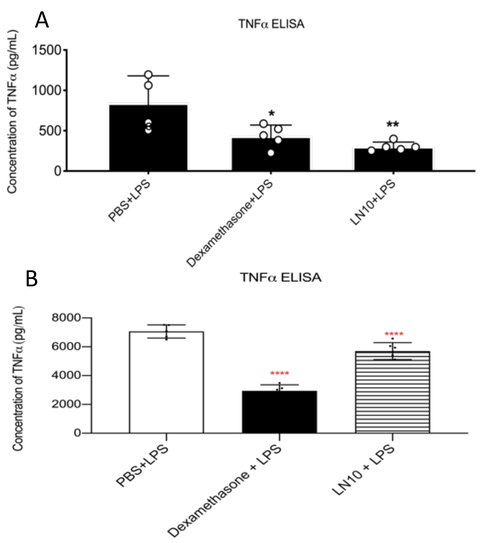

Figure 5: Multifunctional cytokine (TNFα) reduction by LN10 in presence of LPS. (A) Assay performed using fresh PBMCs. (B) Assay performed after 2 months storage of PBMCs at -80C. LN10 maintained its suppressive activity. Consistent reduction of TNFα in both assays, highlights the potential of LN10 as an anti-inflammatory agent.

In summary, we have characterized a lunasin derived peptide (LN10), that does not appear to contain helical structure in solution, in contrast to previous studies, but consistent with the disordered predictions for the full-length peptide. Despite this structural disorder the peptide can suppress TNFα production indicating it might have anti-inflammatory properties. Further study is required to assess the mechanism of action of this peptide which might have implications for understanding the bioactivity and mechanism associated with the full-length peptide.