



Doctoraat Ann Vanhooren: Effects of heat and of near-UV irradiation on goat α -lactalbumin



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Introduction

Proteins have a multitude of roles in biological, medical and industrial processes. However, irradiation with ultraviolet light may reduce or even abolish the biological activity of proteins. Some decades ago it was suggested that the absorption of near-UV light by the aromatic amino acid tryptophan contributes to reductive splitting of disulfides (1). Only recently a few examples of this phenomenon have been registered (2).

We studied the photolysis of disulfide bridges in native and recombinant α -lactalbumins (LA). LA is a small, Ca^{2+} -binding milk protein. LA performs an important function in mammary secretory cells as it is part of lactose synthase.

Structure of α -lactalbumin

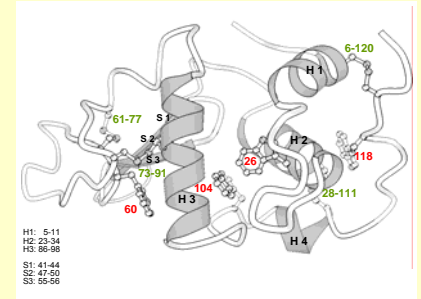


Figure 1: The structure of bovine α -lactalbumin (3) is schematically represented. The location of the major secondary structural elements (S, β -strand and H, α -helix) is highlighted. The four disulfide bridges (green) and the four tryptophans (red) are also shown.

Illumination: set-up

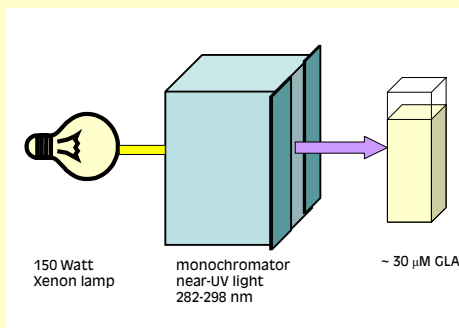


Figure 2: Two and a half ml of a degassed solution was illuminated in a 10 x 10 mm cuvette within an Aminco-Bowman Series 2 spectrofluorimeter (Rochester, N.Y.). The excitation wavelength was centered at 280 nm with a broad bandpass of 16 nm. Ferrioxalate actinometry showed that the incident flux was $6.1014 \text{ photons} \cdot \text{sec}^{-1}$.

Illumination of α -lactalbumin

In goat α -lactalbumin (GLA) illumination with UV-light results in partial unfolding of the protein which can be observed as unusual fluorescence behaviour of the protein.

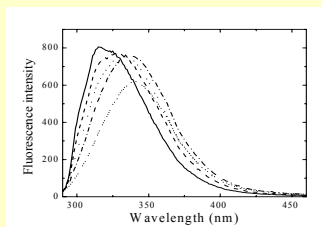


Figure 3: Fluorescence spectra of apo-GLA after different periods of illumination at 280 nm: 0 min (—), 45 min (---), 90 min (· · ·), 180 min (- · -) and 360 min (· · ·).

Prolonged irradiation induces conformational changes diminishing the ability of LA to act as a co-factor in the lactose synthetase complex.

Cleavage of disulfide bonds

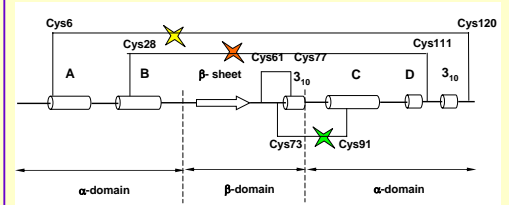


Figure 4: Schematic representation of GLA. Secondary structure elements and the disulfide bonds in native GLA are indicated at the top. Cleaved disulfide bonds are indicated with a coloured star. The domain boundaries are shown as dotted lines.

MALDI and ESI mass spectrometry of the illuminated, carbamidomethylated and digested protein evidences that 3 of the 4 disulfide bonds are lysed by irradiation.

The photolytic reduction of 2 of the bridges leads to the formation of 2 new crosslinks: a Cys6-Lys122 crosslink and a crosslink between either Cys91-Lys79 or Cys73-Lys93.

Illumination of GLA mutants

The impact of the individual Trp residues on the photolysis of disulfide bonds was examined by comparing the photolytic degradation products of each GLA mutant (W26F, W60F, W104F and W118F) with that of wild type GLA.

The fact that no Cys6-Lys122 crosslink and no carbamidomethylated Cys91 was observed in the mutant W26F indicates that the cleavage of Cys6-Cys120 is exclusively mediated by photoexcitation of Trp 26.

The rupture of Cys73-Cys91 seems to be mediated in a direct way by Trp 104.

In all of the recombinant proteins, Cys28-Cys111 resists the mediated photoexcitation, although it is in close contact with Trp 118.

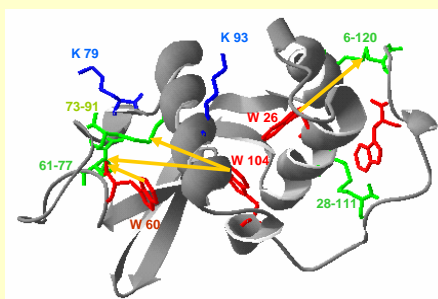


Figure 5: Crystal structure of goat α -lactalbumin (3). The side chains of the four Trp residues (red), the disulfide bonds (green) and two lysine residues (Lys 79 and 93, blue) are represented by sticks. Lys 122 is not represented as it is not possible to define the conformation of residues 121-123 due to the poor definition of the electron density maps in these regions

Finally, our results suggest that Trp60 as well as Trp104, are able to mediate photolysis of disulfide bond Cys61-Cys77.

Conclusions

- Tryptophan residues mediate photoreduction of disulfide bonds
- All Trp residues in GLA contribute to photoreduction, but every Trp residue has its specific impact
- Trp-mediated photoreduction depends on: distance Cys-Trp, strain, involvement of Lys,...
- New Cys-Lys bond is formed

References

- (1) Dose, K., 1968, *Photochem. Photobiol.* 8, 331-335
- (2) Prompers, J. J. et al., 1999, *FEBS Letters* 456, 409-416
- (3) Pike, A.C.W. et al., 1996, *Structure* 4, 691-703