The effect of pH on the structure and function of α-crystallin and Cyclodextrins as artificial molecular chaperones

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Abstract

As the major protein of the lens, α-crystallin is a molecular chaperone that stabilises lens proteins to prevent their precipitation into solution. In this role it is vital in maintaining lens transparency. The chaperone ability of α-crystallin and its individual subunits, αA- and αB-crystallin, has been shown to be sensitive to a variety of environmental and intrinsic factors, including temperature, denaturation and post-translational modification. The effect of pH on α-crystallin chaperone ability, however, has not been thoroughly investigated. There is limited evidence to suggest that the chaperone ability of α-crystallin is pH-sensitive such that α-crystallin is a significantly worse chaperone at pH 6.0 than at pH 8.0. This is of physiological significance since in the lens there is a measurable pH gradient of pH 7.2 in outer lens cells, compared to pH 6.7 in the lens nucleus. A loss of α-crystallin chaperone function in the lens nucleus, as a consequence of decreased pH, may compromise lens transparency. Similarly, extra-lenticular fibrillar aggregation of some disease-related target proteins (Aβ-peptide, for example) is promoted by acidic pH.

This study investigates the effect of pH on the chaperone ability of α-crystallin and its subunits. Further, this study characterises the structural changes to α-crystallin accompanying pH variation in an attempt to explain the structural basis for the observed pH sensitivity. In addition, this study examines the chaperone function of cyclodextrins, a class of chemical chaperones that may act in conjunction with α-crystallin as part of a two-step protein refolding pathway.

Abstract

This study demonstrated that the chaperone activity of α-crystallin is pH sensitive between pH 6.0 and 8.0; the ability of α-crystallin to protect against temperature- and reduction-stress induced amorphous aggregation is significantly reduced at pH 6.0 and 6.5 compared to pH 7.0 and above. The decreased chaperone ability of α-crystallin at pH 6.0 and 6.5 was accompanied by partial unfolding of the protein, and a loss of secondary structure, while α-crystallin quaternary structure remained unchanged. Interestingly, α-crystallin was found to have significant chaperone ability below pH 4.0, conditions under which α-crystallin is largely unfolded. The unfolding of α-crystallin at pH 6.0 and 6.5 is comparatively minor, and it is difficult to say whether this unfolding is directly responsible for the observed pH sensitivity of α-crystallin chaperone ability. The thermal stability of α-crystallin was compromised at pH 6.0 and 6.5, which may partially explain its decreased chaperone ability at these pH values in heat-stress assays conducted at temperatures above 50°C. However, α-crystallin chaperone activity remained pH-sensitive at 37°C and 45°C, at which temperatures it is thermally stable.

Blocking exposed αB-crystallin histidine residues by chemical modification removed, to a large extent, the pH-sensitivity of its chaperone activity. This suggests that the protonation of an exposed histidine residue(s) at pH 6.0 and 6.5 is responsible for the observed pH sensitivity of α-crystallin chaperone ability. Inhibiting the protonation of a specific histidine residue, H83, by site-directed mutagenesis (H83A) did not remove the pH sensitivity of αB-crystallin chaperone activity, and suggests that protonation of this residue alone does not explain the decreased chaperone ability of α-crystallin at mildly acidic pH. This residue lies within the putative chaperone-binding region of αB-crystallin, and is highly

conserved between species and between the human small heat shock proteins. It appears that the protonation of several histidine residues, or residues other than H83, is primarily responsible for the influence of pH on α-crystallin chaperone ability observed in this study. The observed decrease in α-crystallin chaperone function below pH 7.0 partially explains the preferential formation of age-related cataract in the lens nucleus, as the chaperone ability of α-crystallin would be compromised under the mildly acidic conditions characteristic of the nucleus. Additionally, the pH sensitivity of α-crystallin chaperone ability may be significant in the ability of extra-lenticular αB-crystallin to inhibit amyloid-related disease at sites of localised acidosis.

Cyclodextrins are a family of cyclic oligosaccharides that have been shown to function as chemical chaperones under specific protein aggregation conditions. Cyclodextrins have been demonstrated to facilitate the refolding of chemically-stressed target proteins that have already bound to synthetic nanogels, which act in a manner reminiscent of small heat shock proteins. In this study, cyclodextrins were unable to act in conjunction with α-crystallin to facilitate the refolding of thermally-stressed target proteins. β-Cyclodextrin (βCD) demonstrated little or no ability to inhibit the amorphous aggregation of target proteins, but was able to significantly inhibit the fibrillar aggregation of a number of target proteins, including the disease-related A53T α-synuclein mutant. Characterisation of the binding of βCD to target proteins during fibrillar aggregation via circular dichroism, intrinsic and extrinsic fluorescence and competitive chaperone assays provided a model of the cyclodextrin chaperone mechanism. In this model, cyclodextrins interact with already partially unfolded, pre-fibrillar protein intermediates via the insertion of aromatic residues into the cyclodextrin anulus, and by doing so inhibit intra-fibrillar

π-bonding and protofilament assembly. This suggests the potential for cyclodextrins as therapeutic molecular chaperones *in vivo* that may be able to inhibit the pathogenic aggregation of target proteins.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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(Signed)______________________

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List of abbreviations

αCD	α-cyclodextrin
ANS	1-anilo-8-napthalene sulfonate
AS	ammonium sulphate
βCD	β-cyclodextrin
BSA	bovine serum albumin
CD	circular dichroism
Cryo-EM	cryo-electron microscopy
DEPC	diethylpyrocarbonate
DTT	dithiothreitol
ε_{240}	molar extinction coefficient at 240nm
Far UV CD	far ultra-violet circular dichroism
FPLC	fast protein liquid chromotography
γCD	γ-cyclodextrin
GuHCl	guanadinium hydrochloride
IPTG	Isopropyl β-D-1-thiogalactopyranoside
kDa	kilodalton
LB	luria broth
λ_{max}	the wavelength at which maximum fluorescence is observed
L-trp	L-tryptophan
MW	molecular weight
NMWL	nominal molecular weight limit
pKa	acid dissociation constant
PEI	polyethyleneimine

PMSF	phenylmethylsulphonyl fluoride
rcm	reduced and carboxymethylated
ThT	thioflavin T
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoreisis
SEFPLC	size exclusion fast protein liquid chromotography
sHsp	small heat shock protein
TCEP	tris (2-carboxyethyl) phosphine hydrochloride
TEM	transmission electron microscopy
tMax	the time taken to achieve maximum ThT-associated fluorescence
Tris	tris(hydroxymethyl)aminomethane