The binding of small volatile molecules by bovine β-lactoglobulin

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Abstract

Bovine β-lactoglobulin (β-Lg) has been studied extensively but there is no clear identification of its biological function. Hydrophobic molecules have been observed binding into the hydrophobic calyx of β-Lg. By comparison with other members of lipocalin family, it is probable that β-Lg plays a role of transport of ligands, as ligands also bind into the central cavity of lipocalins. The structurally similar MUP is a pheromone-binding protein; therefore, it is possible that β-Lg may also fulfil a similar role. This study has begun to test this hypothesis by investigating the interactions between bovine β-Lg and several small volatile molecules (2-*sec*-4,5-dihydrothiazole, 3-methyl-2-butenal, 3-methyl-2-buten-1-ol and phenylacetic acid). The interactions between the volatile molecules and β-Lg were studied by both two-dimensional NMR spectroscopy and X-ray crystallographic methods. TOCSY spectra were recorded for β-Lg and the complex between β-Lg and the ligands. The observed chemical shifts in the H_N-H_α region are sensitive to the proximity of ligands, and hence chemical shift changes on ligand binding provide information on possible binding sites. It appears that several amino acids with hydrophobic sidechains are affected by interaction with volatile molecules at pH 2.0. The X-ray crystallographic study at pH 8.5 showed that the potential ligand, 2-*sec*-4,5-dihydrothiazole, may have decomposed into a linear 2-methyl-butanol. The refined structure (R=0.281, R_{free}=0.354 for reflections to 2.6 Å resolution) reveals that the potential ligand may bind to the central cavity in a manner similar to the binding of 12-bromodecanoic acid to β-Lg.

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Abbreviations

BBP	Bilin-binding protein
δ	Chemical shift
CD	Circular dichroism
CCP4(i)	Collaborative Computational Project Numer 4 (interface)
COSY	Correlation spectroscopy
COOT	Crystallographic Object Orientated Toolkit
kD	KiloDalton
GUI	Graphical User Interface
HSQC	Heteronuclear multiple quantum coherence
Hz	Hertz
β-Lg	β-Lactoglobulin
α-LA	α-Lactalbumin
MUP	Mouse major urinary protein
3MB	3-Methyl-2-butenal
3MBOH	3-Methyl-2-buten-1-ol
mmol	Millimole
min(s)	Minute(s)
NMR	Nuclear magnetic resonance
NOE	Nuclear Overhauser Enhancement
OBP	Odorant-binding protein
PAA	Phenylacetic acid
RBP	Retinol-binding protein
J	Scalar coupling constant

SDS	Sodium dodecyl sulfate
SBT	2-*sec*-Butyl-4,5-dihydrothiazole
TOSCY	Total correlation spectroscopy

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