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# β-Lactoglobulin interactions with local anaesthetic drugs – Crystallographic and calorimetric studies☆

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ABSTRACT

Interactions between bovine and goat β-lactoglobulin and tetracaine and pramocaine were investigated with isothermal titration calorimetry, X-ray crystallography and molecular modelling. Tetracaine and pramocaine binding to lactoglobulin is an entropy driven endothermic reaction. In this work, we found that determined association constants and thermodynamic parameters indicate that pramocaine has a higher affinity to lactoglobulin than tetracaine.

Crystal structures that were determined with resolutions in the range from 1.90 to 2.30 Å revealed in each case the presence of a single drug molecule bound in the β-barrel in a mode similar to that observed for 14- and 16-carbon fatty acids. The position of the ligand in the β-barrel indicates the optimal fit of 6-carbon aromatic rings to the binding pocket and the major role of hydrophobic interactions in ligand binding. Calculations of tetracaine and pramocaine docking to lactoglobulin revealed that molecular modelling overestimated the role of polar protein–drug interactions.



## 1. Introduction

β-Lactoglobulin (LG) is a protein belonging to the lipocalin family [1]. Lipocalins are often involved in the binding, storage and transport of low-molecular weight hydrophobic ligands [2]. The ability to bind small molecules has made lipocalins an interesting target for utilisation as a new class of drug transporters [3]. Several proteins from this family have been modified and engineered to bind molecular targets with high specificity and selectivity [4].

In β-lactoglobulin, as in other proteins from the lipocalin family, the main structural motif is an antiparallel β-barrel with an entrance surrounded by flexible loops (Fig. 1). The interior of the β-barrel, consisting mainly of non-polar residues, is a preliminary binding site for hydrophobic ligands. The presence of lactoglobulin has been detected in the milk of several mammals, for example cows, goats, sheep, dogs, cats, donkeys, and horses [5]. The biological function of lactoglobulin is not clear; however, this protein is probably involved in the transport of hydrophobic ligands such as fatty acids [6] and retinoids [7].

Bovine lactoglobulin (BLG) is also able to bind other classes of compounds, among them drugs such as chlorpromazine, which is an antipsychotic agent [8], anti-tumour alkaloid ellipticine [9], anthracycline antibiotics such as doxorubicin [10], fluorine-containing drugs [11] and norfloxacin, which is a chemotherapeutic antibacterial drug [12]. Reports are also available on binding several other substances with biological activity such as tea polyphenols [13], serotonin and arachidonoyl serotonin [14], resveratrol [15] and oxali-palladium, which is a promising chemotherapeutic and antineoplastic drug [16]. Despite numerous reports describing the interaction of lactoglobulin with drugs and bioactive molecules, the crystal structures of such complexes remain unknown and there is no structural evidence that compounds containing aromatic rings can be bound in the protein β-barrel.

Local anaesthetic drugs (LA) are compounds that act by interrupting nerve excitation and conduction by direct interaction with voltage-gated channels [17]. Molecules from this group possess a hydrophobic part, usually an aryl-group, and a hydrophilic part containing an amine moiety that is protonable at physiological pH. Both parts are connected by a short alkyl chain and an amide or ester backbone. It has been postulated that the balance between the

*Abbreviations:* LA, local anaesthetic; TET, tetracaine; PRM, pramocaine (pramoxine); LG, lactoglobulin; BLG, bovine β-lactoglobulin; GLG, goat β-lactoglobulin; rmsd, root mean square deviation.

☆ Database accession numbers (PDB): 4Y0P, 4Y0Q, 4Y0R, 4Y0S.

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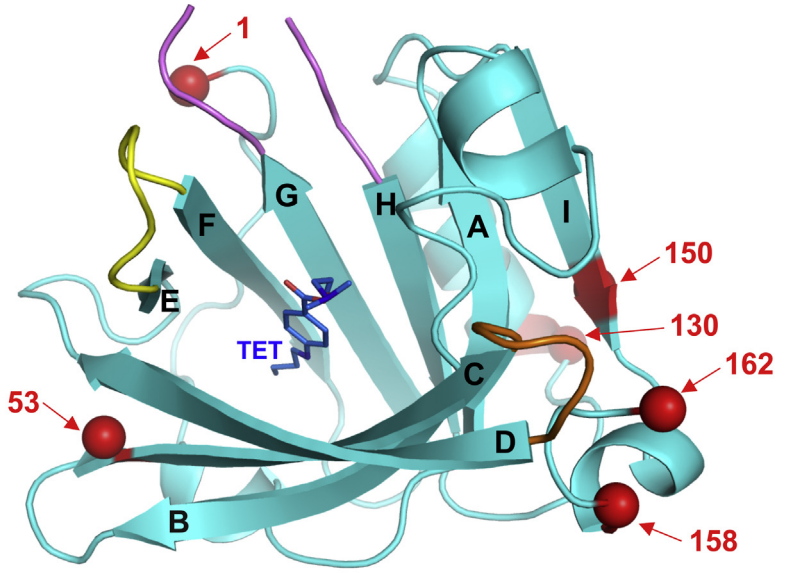


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               10         20         30         40         50
BLG LIVTQTMKGL DIQKVAGTWY SLAMAASDIS LLDAQSAPLR VYVEELKPTP
GLG IIVTQTMKGL DIQKVAGTWY SLAMAASDIS LLDAQSAPLR VYVEELKPTP
    :********* ********** ********** ********** **********
               60         70         80         90         100
BLG EGDLEILLQK WENGECAQKK IIAEKTKIPA VFKIDALNEN KVLVLDTDYK
GLG EGNLEILLQK WENGECAQKK IIAEKTKIPA VFKIDALNEN KVLVLDTDYK
    **:******* ********** ********** ********** **********
               110        120        130        140        150
BLG KYLLFCMENS AEPEQSLACQ CLVRTPEVDD EALEKFDKAL KALPMHIRLS
GLG KYLLFCMENS AEPEQSLACQ CLVRTPEVDK EALEKFDKAL KALPMHIRLA
    ********** ********** *********. ********** *********:
               160
BLG FNPTQLEEQC HI
GLG FNPTQLEGQC HV
    ******* ** *:
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**Fig. 1.** (A) Overall structure of BLG–TET complex and alignment of BLG (isoform B) and GLG sequence.

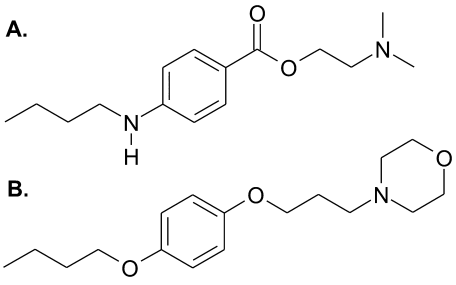


**Fig. 2.** (A) Tetracaine and (B) pramocaine molecule.

hydrophilic and lipophilic parts of the drug molecule influences its potency [18]. Both cationic and uncharged species of LA that coexist at physiological pH could bind to the $Na^+$ channels and block the initiation and propagation of nerve impulses by stabilising the inactivated state of the channels [19].

For our studies, we selected two drugs belonging to the LA group: tetracaine (TET) and pramocaine (pramoxine, PRM). Both drugs have been used as model lactoglobulin ligands to study LG–ligand interactions using X-ray crystallography and isothermal titration calorimetry (ITC). TET and PRM have similar chemical structures consisting of aromatic, aliphatic and polar fragments, elongated shapes and dimensions (Fig. 2) that, as we expected, should fit well with the binding site of β-lactoglobulin (Fig. 3).

Tetracaine belongs to the aminoester family of LA, an important class of nociceptive agents [19], with amphiphilic character [20]. The aromatic ring of TET is soluble in lipids and is important for penetration of a molecule through the lipid bilayer, while the amino group is water soluble and makes it possible to dissolve TET in water. As an amphipathic molecule, tetracaine also has detergent properties [21]. Tetracaine is commonly used in local topical anaesthesia in dentistry and ophthalmology [22]. Pramocaine is less frequently used in medicine than tetracaine. It is a component of some anaesthetic gels used in veterinary practise [23] and anti-itch lotions in medicine [24].

Here, we present a report on interactions between bovine isoform B (BLG) and goat lactoglobulin (GLG) and tetracaine and pramocaine. Both proteins share high sequence and structural similarity and, as lipocalin family members, they are potential candidates for re-engineering and use in medicine as specific drug transporters.

## 2. Materials and methods

### 2.1. Materials

Bovine and goat lactoglobulins were isolated from fresh milk according to a slightly modified method described previously by Neyestani [25] and Blanc [26]. Tetracaine hydrochloride and