Genes Nutr (2007) 2:99–100
DOI 10.1007/s12263-007-0028-3

PROCEEDINGS

# In silico identification of red wine catechin binding sites on human and rat serotransferrins

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Published online: 19 September 2007


**Keywords** Docking · Serotransferrin · Catechin · Homology modelling

## Introduction

Serotransferrin (Tf ) is the protein responsible for the transport of Fe3+ by the blood. Previous ex vivo results showed that Apo A-I and Tf are the principal plasma proteins that bind wine (+)-catechin in humans and rats, respectively [1]. Tf sequence shows two homologous segments of similar length that correspond to the N- and C-terminal half of the protein. These are called as the N- and the C-lobes, respectively (or TfN and TfC). Both lobes have a length of around 350 residues and are connected by a short peptide, and the sequence identity between them is 40%. Tf is able to transport a couple of ions [one Fe3+ and its companion anion (usually CO3 2-)] in each lobe. Structures for proteins that are evolutionarily related with Tf (e.g. lactoferrin) or Tfs from sources other than humans and rats (e.g. rabbits) are known. In all of these, the structure of each lobe is equivalent and consists of two similar domains with a big hole in its interface where the ferric and the carbonate ions bind. When the apo and holo forms of Tf or evolutionarily related proteins are superposed, the binding of the ions is seen to promote a strong conformational change in the protein (i.e. in a lobe the second domain rotates 54° relative to the first domain). No 3D structure is known for Tfrat, whereas only the iron-free (i.e. apo) and the iron-bind (i.e. holo) structures of TfN-human are known. The aims of this in silico study are therefore: (a) to predict the complex between rat Tf and (+)-catechin; (b) to analyse a possible complex between (+)-catechin and human Tf; and (c) to analyse the structure/function relationship in the corresponding complexes and understand, at the molecular level, why (+)-cathechin has an antioxidant activity.

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## Results

No experimental structures are known for ApoTfC-human, HoloTfC-human or for the two lobes of the rat protein in both forms (i.e. ApoTfN-rat, ApoTfC-rat, HoloTfN-rat, HoloTfC-rat). They must therefore be obtained by homology modelling techniques. The structures that will be used as templates for the modelling are selected in accordance with the following criteria: (1) the sequence homology with the sequence to be modelled (this should be as high as possible); (2) the quality of the template (this should also be as high as possible); and (3) the apo and holo templates for modelling apo and holo structures, respectively. Table 1 shows which PDB structures were used (in accordance with these criteria) as templates for modelling the different Tf lobes of unknown experimental structure. Homology modelling was done with the Swiss-Model server (http://swissmodel.expasy.org//SWISS-MODEL.html) [2]. The binding site and the conformation of (+)-catechin into Tfrat were predicted using a blind-docking experiment (i.e.

**Table 1** PDB structures used to model the $Tf_{human}$ and $Tf_{rat}$ lobes of unknown 3D structure

| Modelled lobe | PDB file used as a template | Homology (%) | Resolution (Å) |
|---|---|---|---|
| Apo$Tf_N$ (rat) | 1BP5 (apo $Tf_N$ from human) | 76 | 2.2 |
| Holo $Tf_N$ (rat) | 1A8E (holo $Tf_N$ from human) | 76 | 1.6 |
| Apo $Tf_C$ (rat) | 1CB6 (C-terminal lobe from human apolactoferrin) | 57 | 2.0 |
| Holo $Tf_C$ (rat) | 1H76 (holo $Tf_C$ from pig) | 62 | 2.15 |
| Apo $Tf_C$ (human) | 1CB6 (C-terminal lobe from human apolactoferrin) | 59 | 2.0 |
| Holo $Tf_C$ (human) | 1H76 (holo $Tf_C$ from pig) | 70 | 2.15 |

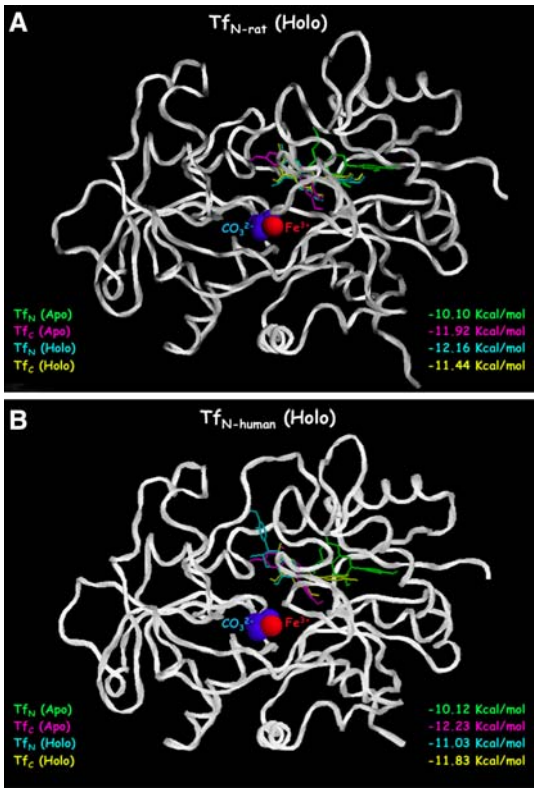


**Fig. 1** Relative location and energy of the highest affinity conformation of (+)-catechin for the different forms (apo or holo) and lobes (Nor C-) of: **a** Tfrat and **b** Tfhuman. The location of the ions and the protein structure from TfN are shown as reference

with no prior definition of the possible binding site). The docking analysis was done with a software package developed in our laboratory called "Blind-docking tester" [3], which extends the capabilities of AutoGrid/AutoDock (http://w3.to/autodock) [4] for this kind of experiment. The results of this docking analysis show a common binding site for (+)-catechin into Tfrat that is independent of the form (apo or holo) and the lobe (N- or C-) (see Fig. 1a). So, when (+)-catechin binds to this common binding site, it blocks the exit of Fe3+ and CO3 2- from the holo forms of Tfrat or blocks the load of these ions by the apo form. The same docking experiment was done with Tfhuman and the results were equivalent to those with Tfrat (see Fig. 1b).

## Conclusions

Previous results from our laboratory showed that (+)-catechin bound to Tfrat but, apparently, showed no preference for Tfhuman [1]. This appears to strongly contradict the results of our present study. It could be argued that most of the in silico results in this study have been obtained using theoretical and not experimental models from the rat and human Tf receptors. However, two of the receptors (i.e. the apo and holo forms from TfN-human) are experimental models, and our present study predicts that both of these receptors are able to bind (+)-catechin with a similar affinity and a similar location to those in Tfrat. This study therefore explains how, at the molecular level, (+)-catechins can play an important role in the prevention of oxidative damage. We show that the binding site in human and rat Tf with the highest affinity for (+)-catechin has a common location in both receptors [irrespective of their source, lobe or form (apo or holo)] and shows a similar affinity for them. This binding site blocks either the delivery of Fe3+ in the holo form or the load of Fe3+ in the apo form. In both cases, the availability of Fe3+ in target cells is inhibited, which contributes to the antioxidant activity attributed to the phenolic compounds in red wine.

**Acknowledgments** This study was supported by grant number CO3/O8 from the Fondo de Investigación Sanitaria (FIS) and AGL2002-00078 from the Comisión Interministerial de Ciencia y Tecnología (CICYT) of the Spanish Government. Montserrat Vaqué is the recipient of a fellowship from grant number CO3/O8.

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