Effect of Cyclodextrins on the ORAC Method for Measuring the Bioactivity of Phytochemicals

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Abstract

Cyclodextrins (CDs) are cyclic oligosaccharides made up by glucose units. Their hydrophobic internal cavity and hydrophilic surface allows them to form inclusion complexes by encapsulating low polar molecules inside. This molecular encapsulation is widely use in biomedicine to improve the water solubility of bioactive compounds. For this reason, CDs have been used to modify the water-based ORAC method to measure the antioxidant activity of lipophilic phytochemicals. However, the literature shows some discrepancy between authors on the role played by these encapsulating agents in the medium. In this work, the effect of cyclodextrins on the ORAC method is investigated in the presence and absence of the antioxidant oxyresveratrol, a hydrophobic stilbene that is naturally synthesised in mulberry trees. By means of a physicochemical and computational approach, it was concluded that cyclodextrins are able to modify the fluorescent signal of the ORAC method both in the presence and in the absence of the antioxidant oxyresveratrol. This interference was dependent on the type of cyclodextrin and the concentration. It seems that the main cause of this undesirable effect is the encapsulation of other reagents in the medium, in particular fluorescein and AAPH. These results are of interest for future studies in which the antioxidant activity of poorly water-soluble biomolecules is analysed.

Introduction

The antioxidant activity of some phytochemicals has been associated with health benefits, such as cancer and obesity prevention, cardioprotection and neuroprotection. Antioxidant activity can be measured *in vitro* by different techniques, but most of them are water-based methods, which makes it difficult to obtain high concentrations of lipophilic phytochemicals. For that reason, some authors modified the ORAC method[1] by adding cyclodextrins (CDs) as solubility enhancers[2] (**Fig. 1B**). CDs are cyclic oligosaccharides with a hydrophobic inner cavity and a hydrophilic outer surface[3]. This structure allows them to form inclusion complexes, encapsulating lipophilic molecules and thus, increasing their solubility in water. However, the literature shows some discrepancy between authors on the role of CDs in this method[4,5]. In this work[6], we evaluate the influence of natural and modified CDs on the ORAC method, by spectrophotometric and bioinformatic assays. Studies were carried out in the presence and absence of the antioxidant oxyresveratrol (**Fig. 1A**) and the potential causes were investigated.

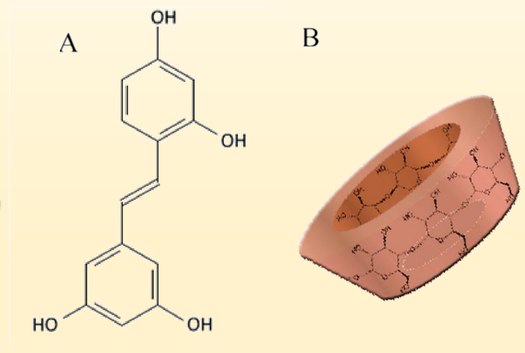

Fig. 1. A) Oxyresveratrol and B) cyclodextrin structures

Effect of CDs on the Assay with and without the Antioxidant

Despite the differences among types of CDs, the value of net AUC of oxyresveratrol increased when these agents were present, especially when β-CD was in the reaction medium (**Fig. 2** – *empty dots*). The highest concentration of β-CD, γ-CD and HP-β-CD was observed to double the net AUC of oxyresveratrol, which could be misinterpreted as antioxidant activity equivalent to twice the oxyresveratrol concentration. However, in the absence of the antioxidant, CDs also increased readings in a dose-dependent manner above a certain concentration that varied according to the type of CD (**Fig. 2** – *filled dots*). Since CDs do not have antioxidant activity, these results suggest that CDs might interfere in the ORAC method, causing a deviation in the measurement of net AUC.

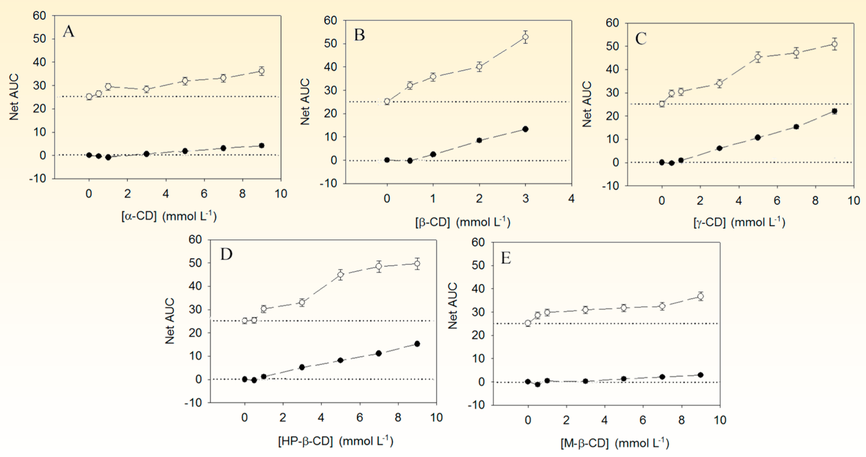

Fig. 2. Effect of increasing concentrations of A) α-CD, B) β-CD, C) γ-CD, D) HP-β-CD and M-β-CD on the ORAC method with (○) and without (●) 2 μmol L^{-1} of oxyresveratrol

Determination of the Origin of the Interference

We evaluate if this interference was because of the glucidic nature of CDs or the encapsulation of other molecules in the medium, such as fluorescein (fluorescent probe) and AAPH (generator of peroxyl radicals). For the first, increasing equivalent concentrations of glucose were analysed in the ORAC method. The results showed a little deviation of ± 5 net AUC that can not fully explain the deviation observed in the readings (**Fig. 3A**). By contrast, the study of the encapsulation of the other reagents revealed that CDs changed both the fluorescence of fluorescein (decrease) (**Fig. 3B**) and absorbance of AAPH (increase) (**Fig. 3C**). It seems that CDs can change the signals of fluorescein and AAPH, which suggests that CDs can encapsulate these reagents along with the antioxidant.

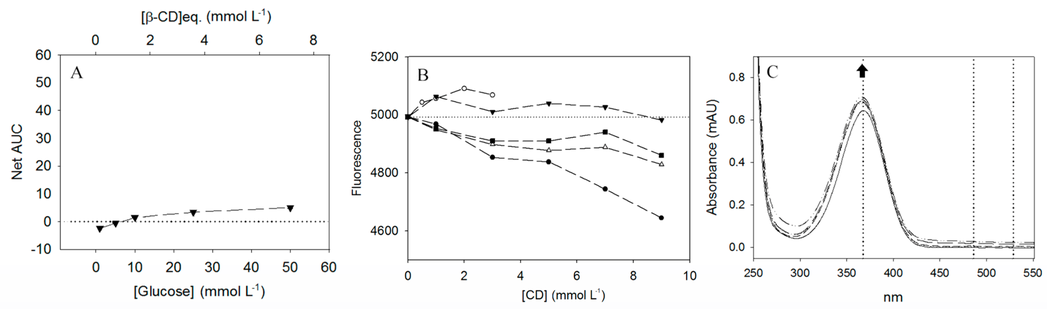

Fig. 3. A) Effect of increasing concentrations glucose on the ORAC method, B) fluorescence signal of fluorescein with (●) α-CD, (○) β-CD, (▼) γ-CD, (△) HP-β-CD and (■) M-β-CD, and C) absorbance spectrum of AAPH with increasing concentrations of β-CD.

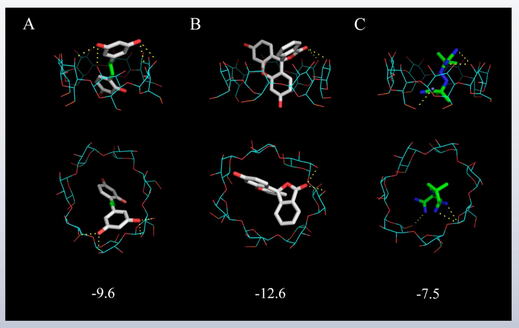

Fig. 4. Molecular docking and scores of β-CD complexes with A) oxyresveratrol, B) fluorescein and C) AAPH. Hydrogen bonds are yellow dotted lines.

Molecular Modelling of the Inclusion Complexes

Since β-CD gave the greatest interference in the method, we performed a molecular docking with this CD as host molecule and oxyresvertarol, fluorescein and AAPH as guest molecules. According to the scores, the higher affinity was observed in fluorescein inclusion complexes, followed by oxyresveratrol and finally AAPH (Fig. 4). In addition, the formation of hydrogen bonds was observed in every configuration. These computational results correlates well with the experimental results displayed above.

Conclusions

The use of CDs as solubility enhancers of lipophilic phytochemicals in the ORAC method is discouraged. The low specificity of these agents have proved to be a problem when they are used in the assay. CDs may interfere in the ORAC method by causing a deviation in the readings that may be related to the encapsulation of other substrates in the reaction medium. By a physicochemical and computational approach, the encapsulation of fluorescein and AAPH is proposed as the reason of this interference. These results are of great interest to avoid erroneous conclusions about the antioxidant activity of phytochemicals.

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