

## Divalent metal ions binding to lactose: a DFT computational study

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In recent years, there has been a growing interest in searching ways to enrich various food products with minerals essential for good health. One of the food products which is important and it is a main part of the people's diet is milk. The main minerals with which the milk is enriched are Ca, Mg, Zn, etc. However, very little information is available about the competition between metals for binding to natural or artificial nutrients in milk. The purpose of this study is to elucidate the factors determining the interactions of lactose, one of the natural ingredients of milk with  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$  cations. DFT calculations of complexes of lactose and  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$  cations at M062X/6-31G(d,p) level of theory are performed. The influence of physicochemical properties, such as ionic radius, preferred coordination and hydration numbers of the metal cation, and influence of the medium on the process of metal binding are estimated.

**Keywords:** lactose, metal binding, calcium, magnesium, zinc.

### INTRODUCTION

Dairy products are a popular part of the diet and are perceived to be healthy. They are a reliable source of calcium, a mineral that the body needs for numerous functions, including building and maintaining bones and teeth, nerve impulses transmission, blood clotting, regulation of the heart's rhythm, etc. The calcium bioavailability of milk is good (about 30 to 35%) and much higher than that of plant foods [1, 2]. The difference in the calcium bioavailability is due to the food composition: milk contains components that act synergistically to promote calcium absorption (lactose, vitamin D, casein phosphopeptides) while plant food contain some inhibitory substances, such as oxalates and phytates.

Food fortification (enrichment) is a strategy for decreasing micronutrient malnutrition at the global level (“A world free from hidden hunger” initiative) by increasing the content of essential micronutrients, i. e. vitamins and minerals (including trace elements) in the food [3, 4]. Milk and dairy products are typically fortified with minerals such as Ca, Mg, Fe and Zn [5, 6]. All minerals used as milk fortificants are considered to be a category “A” risk

as they can interact with each other in terms of absorption and high intakes of one may lead to insufficiency of another [7]. The negative effect of high dietary intakes of calcium and phosphorus on zinc absorption as a result of interactive effects has been demonstrated [6]. Zinc is essential for many basic physiological functions; the human body has a limited zinc storage capacity and zinc deficiency can develop very rapidly when intakes are low. A potential mechanism by which calcium interferes with zinc absorption is competition for a divalent cation channel across the brush border membrane [8, 9]. On the other hand calcium levels in the human body have to be balanced with magnesium: adequate levels of magnesium are needed in order to properly use calcium; magnesium deficiency affects calcium metabolism and alters levels of certain hormones that regulate calcium in the body. Several studies have reported that calcium and magnesium intakes influence each other's absorption [10, 11]. Very little information is available about the competition between metals for binding to natural or artificial nutrients in milk [12]. The goal of this paper is to shed light on this issue and examine the interactions between lactose, naturally occurring sugar in milk, which is used as fortificant in some fortified milks for children [13], and calcium, magnesium and zinc ions (also naturally occurring and/or added as mineral fortificants to milk). The sub-aims of the pre-

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sent study are: (a) to clarify whether and to what extent the properties of the metal ion govern the metal binding affinity and selectivity for lactose; (b) to assess the role of external factors such as the dielectric properties of the medium in the process of metal binding and competition in these complexes. In achieving these aims, we conducted DFT calculations combined with the PCM (Polarizable Continuum Model, one of the most widely used implicit continuum solvation model) computations for the complexes resembling  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$  ions binding to lactose.

## COMPUTATIONAL DETAILS

The molecules of galactose, glucose, bare and hydrated metal cations  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$  and their complexes were optimized using the Gaussian 09 program package [14]. The calculations were performed at M062X/6-31G(d,p) level of theory. This combination method/basis set was chosen because it reliably reproduces the metal – oxygen bond distances in the 1:2 Ca-lactose complex: the M062X/6-31G(d,p) calculated  $\text{Ca-O}_{\text{lactose}}$  average distance, 2.506 Å, is very close to the experimental one – 2.500 Å [15]. All optimized structures were verified to be minima of the potential energy surface by means of frequency calculations. No imaginary frequencies were found for any of the structures. The differences between the products and reactants of electronic energies,  $\Delta E_{\text{el}}$ , thermal energies, including zero-point energy,  $\Delta E_{\text{th}}$ , and entropies,  $\Delta S$  were used to evaluate the gas-phase free energy of the complex formation,  $\Delta G$ , at  $T = 298.15$  K according to the equation ( $P\Delta V$  below is a work term):

$$\Delta G = \Delta E_{\text{el}} + \Delta E_{\text{th}} + P\Delta V - T\Delta S \quad (1)$$

The influence of the solvent was estimated by PCM (Polarizable Continuum Model) calculations [16, 17] as implemented in the Gaussian 09 [14]. All the structures were fully optimized in water environment ( $\epsilon \approx 78$ ) and the respective  $\Delta E_{\text{el}}^{78}$ ,  $\Delta E_{\text{th}}^{78}$  and  $\Delta S^{78}$  were used to evaluate the free energy in solution,  $\Delta G^{78}$  (following eq. 1).

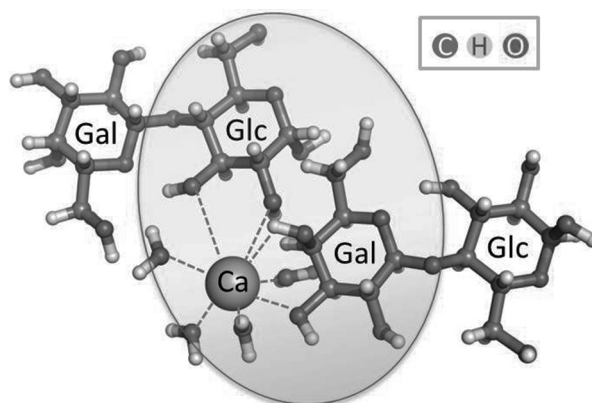
The complex formation is thermodynamically favorable if the calculated value of  $\Delta G$  is negative, and the process is unfavorable if the value of  $\Delta G$  is positive.

All the calculations reported herein are corrected for basis set superposition errors (BSSE) using the counterpoise procedure of Boys and Bernardi [18] as coded in Gaussian 09 package [14]. The PyMOL molecular graphics system was used in generating the molecular graphics images [19].

## RESULTS AND DISCUSSION

**Ca-lactose complex:** The crystal structure of the hydrated calcium bromide complex of lactose (4-O- $\beta$ -D-galactopyranosyl-D-glucopyranose) (Fig. 1; [15]) was taken as a basis for our modeling study (see below). In the experimental structure the calcium ion binds two lactose molecules and four water molecules [15]. First lactose molecule is coordinated to the calcium ion through two oxygen atoms of its galactose moiety and the second one is coordinated through two oxygen atoms of its glucose unit. There are no close contacts between calcium cations and bromide anions (the closest bromide-calcium distance is about 5 Å) [15].

Note that the galactose moiety of the first lactose molecule and the glucose moiety of the second one are not connected directly to the metal and, apparently, do not contribute significantly to the energetics of the complex formation, if at all. Thus, the surrounding of  $\text{Ca}^{2+}$  can be represented in a simplified manner by using a model in which calcium ion



**Fig. 1.** Environment of the calcium ion in the Ca-lactose complex [15]. Gal = galactose, Glc = glucose.

binds to one galactose molecule, one glucose molecule and four water molecules (Fig. 2). Thus, after omitting the non-metal-coordinating sugar moieties, the immediate surrounding of the calcium cation is preserved the same as in the crystal structure above (Fig. 1): the metal coordination shell is composed of four water molecules and two sugar rings. The resultant construct was fully optimized where the  $\text{Ca}^{2+}$  cation is octacoordinated to the surrounding ligands (Fig. 2) with metal-oxygen bond distances  $\text{Ca}^{2+}\text{-O}_{\text{glucose/galactose}}$  in the range  $2.43 \div 2.69$  Å.

**Mg- and Zn-lactose model system complexes:**  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$  cations in the respective complexes

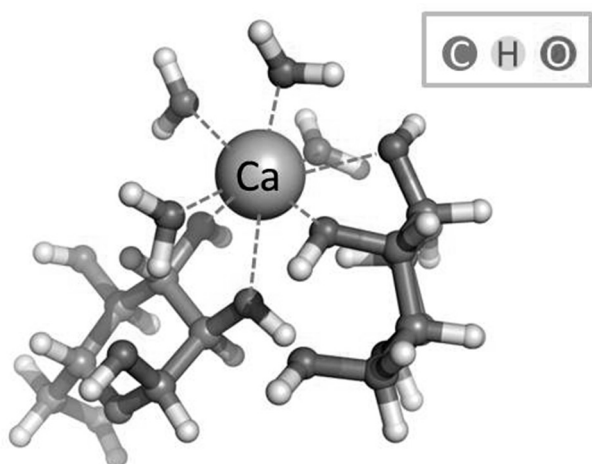


Fig. 2. Fully M062X/6-31G(d,p) optimized structure of the Ca-complex.

were located at the same position as the  $\text{Ca}^{2+}$  cation in the model system (Fig. 2). The ligand surrounding of the metal entities was also kept the same. The systems were allowed to fully relax upon geometry optimization. The resultant optimized structures of the respective metal complexes are shown in Figure 3. Note that the coordination number of  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$  cations in the complex was reduced to 6 as the two sugar moieties bind the metal in a monodentate fashion (Fig. 3).

Thermodynamic parameters for the reaction  $\text{Gal} + \text{Glc} + \text{M}^{2+} + n\text{H}_2\text{O} \rightarrow [\text{M-complex}]^{2+}$  with bare metal cations are given in Figure 3. Data presented indicate that there is a rough correlation between the metal cation radius and  $\Delta G$  values: the smaller the cationic radius is (and higher is the respective charge density of the cation), the more thermodynamically favorable is the complex formation. Ionic

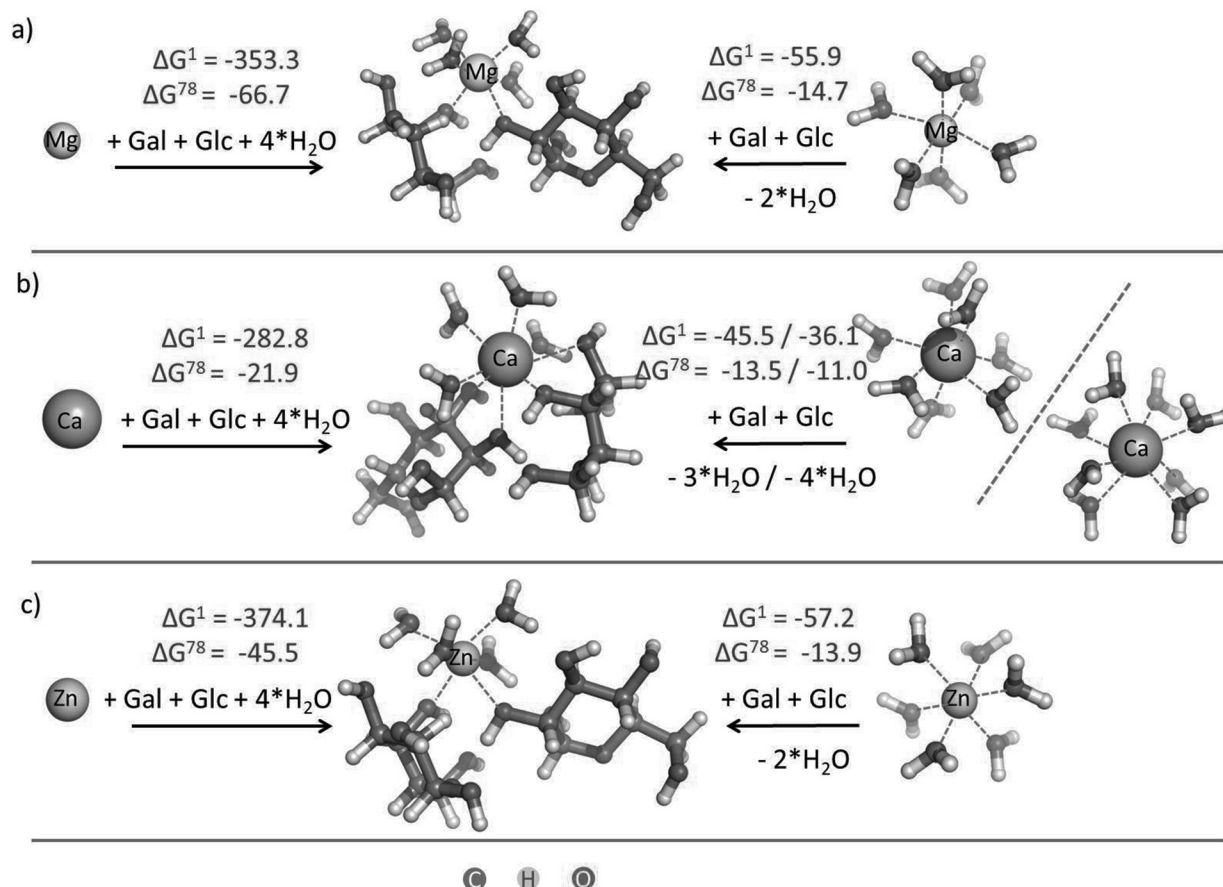


Fig. 3. M062X/6-31G(d,p) optimized structures of  $\text{Mg}^{2+}$  (a),  $\text{Ca}^{2+}$  (b) and  $\text{Zn}^{2+}$  (c) cations bound to a model lactose (middle). The free energies  $\Delta G^1$  and  $\Delta G^{78}$  (in kcal/mol) for the complex formation reactions for non-hydrated (left-hand side) and hydrated metal (right-hand side) cations are shown.  $\Delta G^1$  refers to reaction free energy in the gas phase, whereas  $\Delta G^{78}$  refers to reaction free energies in an environment characterized by an effective dielectric constant of 78 (water).

radii of metal cations and their coordination numbers are presented in Table 1.  $Mg^{2+}$  and  $Zn^{2+}$  cations in octahedral configuration have smaller ionic radii than  $Ca^{2+}$  and the reactions of the complex formation with these cations are characterized by lower  $\Delta G$  values than their  $Ca^{2+}$  counterparts. The higher Lewis acidity of  $Zn^{2+}$ , as compared to  $Mg^{2+}$  and  $Ca^{2+}$ , also enhances its competitiveness with respect to its rivals. Calcium cation has a larger ionic radius and the respective  $\Delta G$  values for the  $[Ca\text{-complex}]^{2+}$  formation are higher.

**Table 1.** Effective ionic radii of metal cations (Å) [20]

Cation	Coordination	Ionic radius <sup>a</sup>
$Mg^{2+}$	VI	0.72
$Ca^{2+}$	VII	1.06
$Ca^{2+}$	VIII	1.12
$Zn^{2+}$	VI	0.74

All of the reactions of the complex formation with bare metal cations are characterized with quite large negative formation free energies in the gas phase. Solvation effects, however, greatly attenuate the free energy gains in the gas phase although the reactions are predicted to be still exergonic (occur spontaneously) in aqueous medium. Since the solvation effects seem to play a significant role in the process, we increased the level of modeling and incorporated water molecules around the cations to take explicitly into account the first hydration shell: metal ions in aqueous solution are hydrated. The structures of the hydrated metal ions in aqueous solution can display a variety of configurations depending on the size and electronic properties of the metal ion. The metal ions with an ionic radius in the range 0.55–0.98 Å are expected to be octahedral, while those with an ionic radius larger than 0.98 Å are expected to have higher coordination numbers [21]. Indeed, the experimentally found hydration numbers and configurations for the studied metals are as follows: for  $Mg^{2+}$  and  $Zn^{2+}$  – coordination number 6 and regular octahedral configuration; for  $Ca^{2+}$  – coordination numbers 7 or 8 and capped octahedron or square antiprismatic configurations, respectively [22, 23]. These basic configurations of the hydrated metal ions ( $[Mg(H_2O)_6]^{2+}$ ,  $[Ca(H_2O)_7]^{2+}$ ,  $[Ca(H_2O)_8]^{2+}$ ,  $[Zn(H_2O)_6]^{2+}$ ) in aqueous solution are used to re-model the reaction of complex formation with lactose.

Calculations demonstrate that hydration of the metal cations significantly increase the reaction free energies making the process of the ligands-

(hydrated metal cation) complex formation less favorable than that of the respective ligands-(bare metal cations) counterparts. The metal binding to the ligands, however, is still favorable as evidenced by the negative  $\Delta G^1$  and  $\Delta G^{78}$  values in Figure 3. Among the three metal cations, the complex formation with  $Mg^{2+}$  and  $Zn^{2+}$  cations appears to be more advantageous than that of the  $Ca^{2+}$  complexes (more negative  $\Delta G^1$  and  $\Delta G^{78}$  values for the former than the latter). The reactions with hepta- and octahydrated  $Ca^{2+}$  cations are characterized with different free energies as the difference in  $\Delta G^1$  values is more pronounced. The model with implicit and explicit water consideration gives  $\Delta G^{78}$  values for the three metal cations studied in the range of  $-14.7$ – $-11.0$  kcal/mol.

## CONCLUSIONS

By employing density functional theory (DFT) calculations at M062X/6-31G(d,p) level combined with continuum dielectric method (PCM) computations, the thermodynamic descriptors ( $\Delta G^1$  and  $\Delta G^{78}$ ) of the metal ( $Ca^{2+}$ ,  $Mg^{2+}$  and  $Zn^{2+}$ ) binding to a model representing lactose have been evaluated and the interaction between the binding partners assessed. The DFT calculations confirm the experimental findings [8] that  $Ca^{2+}$  ion binds favorably to lactose as the free energy of the complex formation is negative.  $Mg^{2+}$  and  $Zn^{2+}$  can successfully compete with  $Ca^{2+}$  ion for the lactose binding as evidenced by the negative (and lower than those for  $Ca^{2+}$ )  $\Delta G^1$  and  $\Delta G^{78}$  values.

The calculations reveal few key factors governing the process of  $Ca^{2+}$ ,  $Mg^{2+}$  and  $Zn^{2+}$  cations binding to a lactose model system with omitted non-metal-coordinating sugar moieties:

- The physicochemical properties of the metal cation – its ionic radius, coordination and hydration number, and Lewis acidity;
- The dielectric properties of the medium.

It can be assumed that naturally occurring and/or added as fortificants to milk  $Ca^{2+}$ ,  $Mg^{2+}$  and  $Zn^{2+}$  cations and lactose can interact in a competitive manner.

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## ИЗСЛЕДВАНЕ С ТЕОРИЯ НА ФУНКЦИОНАЛА НА ПЛЪТНОСТТА НА СВЪРЗВАНЕТО НА ДВУВАЛЕНТНИ МЕТАЛНИ ЙОНИ С ЛАКТОЗА

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(Резюме)

През последните години нараства интересът към търсене на начини за обогатяване на различни хранителни продукти с минерали, които са полезни за здравето. Един от хранителните продукти, който е важен и е основна част от диетата на хората е млякото. Основните минерали, с които се обогатява млякото са Ca, Mg, Zn и др. Въпреки това все още има малко информация за конкуренцията на свързването на металите с изкуствените и естествени съставки на млякото. Целта на това изследване е да се изяснят факторите, определящи взаимодействието на лактозата, една от естествените съставки на млякото, с Ca<sup>2+</sup>, Mg<sup>2+</sup> и Zn<sup>2+</sup> катиони. Направени са изчисления с теория на функционала на плътността на комплекси на лактоза и Ca<sup>2+</sup>, Mg<sup>2+</sup> и Zn<sup>2+</sup> катиони на ниво M062X/6-31G(d,p). Оценено е влиянието на физикохимичните свойства на металните катиони, като йонен радиус, предпочитано координационно и хидратационно число, оценено е и влиянието на средата върху процеса на метално свързване.