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Digest paper

Are enzymes sensitive to the multivalent effect? Emerging evidence with glycosidases

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ABSTRACT

The multivalent effect in glycosidase inhibition is rapidly emerging as a hot topic of the research. In the last five years, several iminosugar-based inhibitors showed remarkable multivalent effects towards specific glycosidases, thus demonstrating that the concept of multivalency goes far beyond the interaction among protein receptors, as lectins, and carbohydrates and can be involved also for enzymes bearing a single active site. This critical overview aims to illustrate and discuss both cases of glycosidases prone to accept inhibitors with a multivalent presentation of the bioactive molecule as well as enzymes displaying a controversial behavior.

Contents

Introduction

This review aims to highlight in a critical way the advances in the field of glycosidase inhibition by using multivalent iminosugars, since 2009, when the first example suggesting a multivalent effect for such enzymes has been reported [1]. Particular emphasis will be devoted to glycosidases of therapeutic interest.

The multivalent effect (MVE) is generally defined as the increase of the biological response observed for compounds possessing more than one bioactive units linked/connected to a common scaffold compared to the sum of the contributions given by the individual bioactive molecules, as represented in Fig. 1.

A clear and unambiguous definition of the multivalent effect is necessary, since the simultaneous presentation of multiple binding units may enhance the relative potency (rp) of the multivalent architectures with respect to the monovalent ligands simply as a consequence of an increase in local concentration. However, it is

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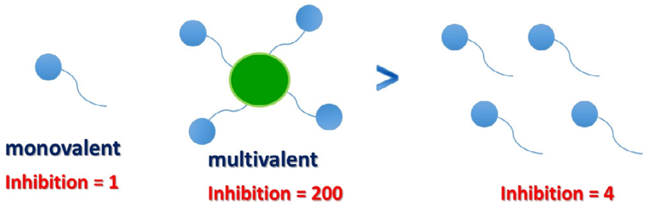

Fig. 1. Cartoon representation of the multivalent effect (MVE).

becoming clear that effectiveness of a ligand can enhance when presented in a cluster rather than alone. Winum, Ulrich and co-workers [2] defined the *positive multivalent effect* as arising when the relative potency of the ligands is higher in the multivalent system compared to the monovalent one. Taking into account that only multivalent entities presenting considerable MVE are of interest for biological applications, we will address, in this review, only those systems showing *positive MVEs*, evaluated on the basis of the relative potency per binding unit (rp/*n*). This value is given by the ratio of the relative potency rp (K_i or IC_{50} of the multivalent compound over the monovalent one) and the valency *n* (the number of bioactive units within the system). If rp/*n* is higher than 1, a positive multivalent effect is considered to be effectively taking place.

From the practical point of view, a real advantage of dealing with a multivalent ligand (that often requires lengthy synthesis) may arise only when the same biological response (e.g. inhibition potency) is not achievable by increasing the concentration of the monovalent compound.

This effect has only recently become a "hot topic" of the research in the field of glycosidase inhibitors, while it has been extensively investigated concerning the interaction of lectins with carbohydrates [3]. Nevertheless, the multivalent binding modes are far to be unequivocally elucidated. The generally accepted phenomena which may account for the observed MVE are summarized in Fig. 2, and comprise: 1) *Statistical rebinding effect* (Fig. 2A), due to the enhancement of the local concentration of the active molecule in proximity of the binding site; 2) *chelate effect* (Fig. 2B), involved only when the enzyme presents more than one catalytic site; 3) *subsite binding effect* (Fig. 2C), that may determine either a stronger binding of the inhibitor by non specific interactions with non-catalytic subsites (Fig. 2C/a) or a steric hindrance that hampers access of the substrate to the catalytic site (Fig. 2C/b); 4) *clustering effect*, when the association of several units of enzyme is favored over one multivalent molecule (Fig. 2D/a), or with the formation of a cross-linked network if the enzyme also possesses a multimeric form (Fig. 2D/b) [2,4].

Iminosugars, that are nitrogenated glycomimetics in which the carbohydrate endocyclic oxygen is replaced by a nitrogen atom, have been recognized for a long time as potent glycosidases inhibitors [5]. Many iminosugars display high affinity towards the active site of glycosidases, thus making them ideal candidates for the construction of multivalent architectures. Several different types of scaffolds have been reported for building multivalent iminosugar compounds, ranging from simple dendrimers, to cyclodextrins, fullerenes and calixarenes and, indeed, the few reviews [4] appeared on related topics are mainly organized by the nature of the scaffold or its valency [4b,c]. However, in contrast to the wide variety of the explored scaffolds, only few active molecules (basically deoxynojirimycin, DNJ, and deoxymannojirimycin, DMJ, Fig. 3) have been demonstrated active in their multimeric fashion. This is probably due to the inherent challenges connected to the synthesis of new iminosugar compounds, highly hydrophilic molecules for which the standard chemical and purification methods of organic compounds do not often apply. Probably, for the above mentioned reasons, reviews on multivalent based nanoconstructs generally do not address only iminosugar based ones [4a–b,6].

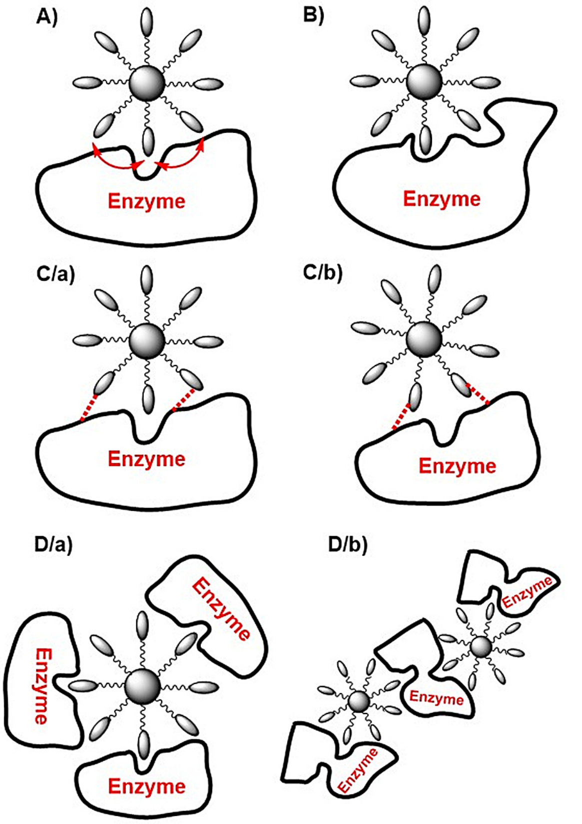

Fig. 2. Proposed binding models accounting for the multivalent effect: A) *Statistical rebinding effect*; B) *Chelate effect*; C/a) *Subsite binding effect* deriving from interactions with non-catalytic subsites; C/b) *Subsite binding effect* due to steric hindrance; D/a) *Clustering effect* with more than one enzyme units; D/b) *Clustering effect* with multimeric enzymes leading to the formation of cross-linked networks.

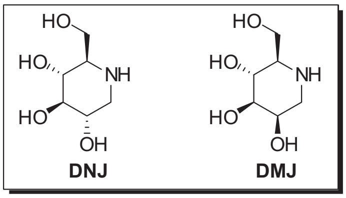

Fig. 3. Structures of deoxynojirimycin (DNJ) and deoxymannojirimycin (DMJ).

Unlike already published reviews [2,4a], and taking into account different multivalent compounds and a larger variety of enzymes, this account is focused on inhibitory activities of multivalent iminosugars against glycosidases, with the aim to help the organic synthetic chemists in a critical evaluation for the future design of new inhibitors that will benefit from their multivalent presentation in terms of inhibition or selectivity for a specific enzyme. Unfortunately, very few human enzymes are available at the moment and most of the reported examples investigate the MVE by using commercial glycosidases as models on the basis of their structural analogies with the human ones (such as Jack Bean α-mannosidase). The screening of therapeutically relevant enzymes still remains an urgent and highly desirable goal.

A classification by glycosidic enzyme type is considered here: this organization allows to highlight receptors that are more prone to accept compounds with a multivalent presentation and those that are not.