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Detoxification of alkyl methylphosphonofluoridates by an oxime-substituted β-cyclodextrin – An in vitro structure–activity study

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HIGHLIGHTS

- The ability of a β-cyclodextrin derivative to detoxify 11 sarin derivatives was assessed.
- Detoxification is always faster than hydrolysis of the respective organophosphonate.
- A structure–activity relationship was established.
- Rates were correlated with stabilities of the respective cyclodextrin complexes.

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ABSTRACT

Detoxification rates of a series of alkyl methylphosphonofluoridates by an oxime-substituted β-cyclodextrin (β-CD) were assessed quantitatively by using an AChE inhibition assay. The cyclodextrin (CD) derivative was identified in previous work as a highly active cyclosarin scavenger. Here, a structure–activity relationship was established by investigating the effect of this CD on the detoxification of sarin derivatives differing in the structure of the alkoxy residue. The results show that detoxification rates correlate with the steric bulk and chain length of the alkoxy group in the organophosphonate (OP). OPs with larger, more bulky residues are detoxified more rapidly, with the exception of soman, which is bearing a pinacolyloxy side chain. In addition, the substituted CD was in every case more active than unsubstituted, native β-CD with up to a 400-fold difference. Comparing the kinetic results obtained with the known thermodynamic stabilities of related β-CD complexes indicate that detoxification rates generally increase when the alkoxy residue on the OP is exchanged by a residue, which forms a more stable complex with β-CD. This correlation lends support to the proposed mode of action of the substituted CD, involving initial complexation of the OP followed by reaction between the CD and the OP. The moderate to high efficacy on the detoxification of sarin derivatives suggests the potential applicability of this CD as a small molecule scavenger for G-type nerve agents.

1. Introduction

Every year 2–5 million people suffer from poisonings by pesticides (accidental or suicidal) and up to 220,000 fatal cases are estimated (Karalliedde and Szinicz, 2001). Pesticides are a heterogeneous group of synthetic chemicals that include organophosphorous compounds (OP) whose toxicity is due to the covalent modification and the resulting inhibition of the enzyme acetylcholinesterase (AChE) (Taylor et al., 1995). AChE inhibition causes accumulation of the neurotransmitter acetylcholine leading to disturbance of numerous body functions and eventually to death due to respiratory arrest. Standard treatment of OP poisoning includes an anti-muscarinic compound, e.g. atropine, and an oxime as AChE reactivator (Wiener and Hoffman, 2004). However, numerous limitations are associated with standard treatment protocols. Antimuscarinic drugs do not counteract OP effects at nicotinic receptors, for example. Moreover, oximes hardly cross the blood–brain-barrier (Eyer and Worek, 2007; Aurbek et al., 2009) and are generally insufficient in reactivating AChE inhibited by certain nerve agents, e.g. soman and tabun (Eyer and Worek, 2007; Worek et al., 2004). Also reactivation by oximes of inhibited AChE containing an aged OP residue resulting from hydrolysis of an alkoxy residue on the phosphorus atom is not feasible (Aldridge and Reiner, 1972; Worek et al., 1996). Finally, treatment of

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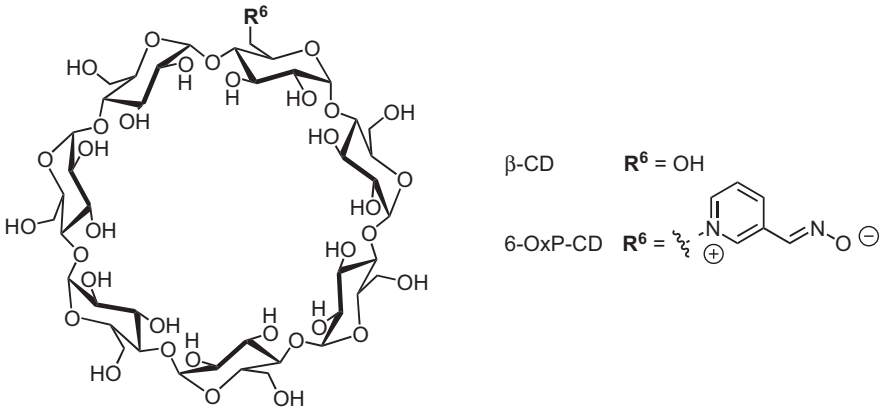

Fig. 1. Chemical structures of β-CD and 6-OxP-CD.

poisonings with persistent nerve agents such as VX that are only slowly cleared from the organism is particularly difficult (Reiter et al., 2008, 2011; Tenberken et al., 2010). In order to overcome these limitations, new therapeutic approaches are urgently needed. One research effort is directed at the development of stoichiometric or catalytic enzymes that can act as scavengers by binding or degrading OPs *in vivo* prior to the appearance of any toxic effects (Masson and Rochu, 2009). Synthetic compounds, so-called small molecule scavengers, can serve similar purposes.

Promising lead structures for the development of small molecule scavengers are cyclodextrins (CDs; Fig. 1), cyclic oligosaccharides containing glycopyranose subunits linked by α(1–4) glycosidic linkages. Negligible toxicity and the relative ease with which CDs can be structurally modified led to application of this class of compounds in many different areas (Szejtli, 1998). A characteristic feature of CDs is their ability to include small hydrophobic compounds in the apolar interior of the macrocyclic cavity. Stability of the complexes formed depends on ring size of the CD and on the structure of the substrate. In general, substrates that efficiently occupy the available space of the CD cavity are bound best. In the case of the seven-membered β-cyclodextrin (β-CD), for example, adamantane derivatives are very good substrates (Rekharsky and Inoue, 1998). Complex stability is determined by a combination of intermolecular interactions of which the most important are vander-Waals interactions (Bergeron et al., 1978), hydrogen bonding (Tabushi et al., 1978), dipole–dipole interactions (Gelb et al., 1981; Bergeron et al., 1977), and the hydrophobic effect (Connors, 1997).

Several studies have shown that native CDs can also degrade the nerve agents soman and sarin (Hennrich and Cramer, 1965; Van Hooidonk and Groos, 1970; Desiré and Saint-André, 1987). Degradation efficiency could be improved by arranging functional groups around the CD cavity that actively participate in the reaction. Examples include functionalization of β-CD with 2-iodosobenzoates or oximes, which afforded scavengers for cyclosarin, tabun, or paraoxon (Masurier et al., 2005; Wille et al., 2009; Le Provost et al., 2011; Zengerle et al., 2011). Zengerle et al. (2011) showed that β-CD derivative 6-OxP-CD (Fig. 1) with a pyridinium oximate in 6-position of one glucose unit detoxifies the toxic (−)-enantiomer of cyclosarin within seconds and the less toxic (+)-enantiomer with a half-time of 43 s under appropriate conditions. However, the same CD had only a small effect on tabun detoxification (Brandhuber et al., 2012).

In order to investigate whether detoxification activity of 6-OxP-CD is limited to cyclosarin or if this compound is able to detoxify other alkyl methylphosphonofluoridates with similar efficiency, rates of 6-OxP-CD-mediated detoxification of a series of sarin derivatives differing in the alcohol component (Fig. 2) were assessed by using an enzymatic *in vitro* assay. These measurements allowed establishing a structure–activity relationship demonstrating that sarin derivatives with larger, bulky alkyl substituents that presumably interact with 6-OxP-CD more strongly are detoxified more rapidly than OPs with small alkyl residues.

2. Materials and methods

2.1. Materials

Acetylthiocholine iodide (ATCh), β-cyclodextrin, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) and tris(hydroxymethyl)aminomethane (TRIS) were obtained from Sigma–Aldrich (Taufkirchen, Germany). Disodium hydrogenphosphate dihydrate, potassium dihydrogenphosphate, and hydrochloric acid were purchased from Roth (Karlsruhe, Germany). Sarin, cyclosarin, soman, ethylsarin, *n*-propylsarin, *iso*-butylsarin, *n*-butylsarin, *iso*-pentylsarin, *n*-pentylsarin, *neo*-pentylsarin and *sec*-pentylsarin (Fig. 2; >95% by GC–MS, ^{1}H NMR and ^{31}P NMR) were made available by the German Ministry of Defence (Bonn, Germany). 6-OxP-CD was synthesized according to Zengerle et al. (2011). All other chemicals were purchased from Merck Eurolab (Darmstadt, Germany).

Stock solutions of sarin, soman, cyclosarin (0.1%, v/v), and sarin analogs (1%, v/v) were prepared in acetonitrile, stored at room temperature, and diluted appropriately with Tris–HCl buffer (0.1 M, pH 7.4) just before use. CD stock solutions (10 mM) were prepared daily in Tris–HCl buffer and diluted as required with Tris–HCl buffer prior to use.

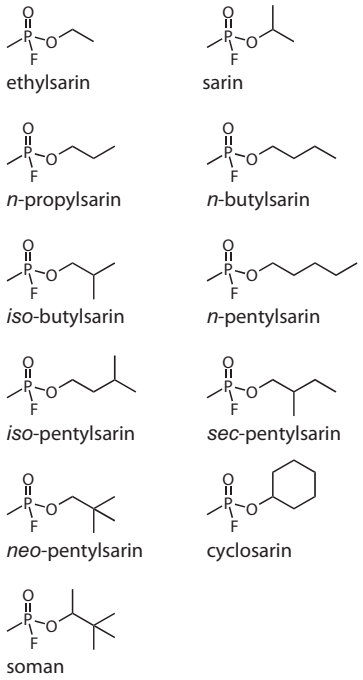

Fig. 2. Chemical structures of the alkyl methylphosphonofluoridates tested.