Hofmeister ions series protected protein during contact with organic interface

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INTRODUCTION

The association between chitosan and liposomes obtained by Reverse phase evaporation method (REVs-Chi) stabilized by polyvinylic alcohol (PVA) is proposed as a controlled delivery for Diphtheria toxoid (Dtxd). During this process, the protein suffers stresses with the organic solvent contact and the strong agitation.

Because preparation method characteristics a pre-formulation study on the Dtxd structure is shown.

The protein integrity after sonication with ethyl acetate in the presence of Hofmeister ions series salts was followed by HPLC[2,3], CD, ELISA and fluorescence[3].

MATERIAL AND METHODS

Materials:

Dtxd (Instituto Butantan); all salts and organic solvents were of analytical grade.

Methods:

REVs-Chi preparation:
Briefly, the Dtxd in 0,5 % chitosan was added to soy phosphatidylcholine/cholesterol/ethyl acetate. After micelle formation, by sonication, the formed organogel was resuspended in PBS or 1% PVA[1].

Dtxd stability:
The Dtxd was diluted in KSCN; NaH_2PO_4; NaCl or $MgCl_2$ (0 to 150 mM salt) at fixed protein concentration (85,6 µM), and than added to a fixed volume of $CH_3CO_2C_2H_5$ (ethyl acetate). Immediately the moistures were sonicated at 40 W during 4 minutes. The emulsions were centrifuged and the aqueous phases containing the soluble protein were analyzed by HPLC[2,3], ELISA, UV, fluorescence and CD spectroscopies.

RESULTS AND DISCUSSION

Analysis of Dtxd solubility and immunological identity

Dtxd remained 98% soluble in the presence of PO_4^{2-} and decreased (5-40 %) in the presence of Cl^-, SCN^- and Mg^{2+} **(Figure 1).**

PO_4^{2-} retained the Dtxd conformation immunologically recognized **(Figure 2).**

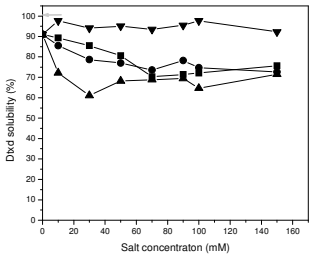

Figure 1. Effect of Hofmeister ion series salts in the Dtxd solubility. The protein's concentrations were calculated by absorbencies at 280 nm in the presence of (-■-) NaCl, (-●-) KSCN, (-▲-) $MgCl_2$ e (-▼-) NaH_2PO_4, after sonication with ethyl acetate ($CH_3CO_2C_2H_5$), by UV spectroscopy. Control: not sonicated Dtxd in water (→).

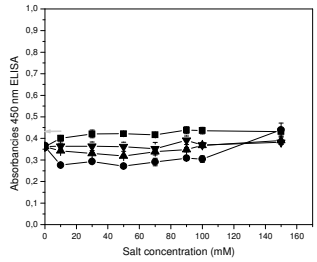

Figure 2. Effect of Hofmeister ion series salts in the immunological Dtxd identity after sonication. The Dtxd soluble fractions in the presence of (-■-) NaCl, (-●-) KSCN, (-▲-) $MgCl_2$ e (-▼-) NaH_2PO_4. Control: not sonicated Dtxd in water (→).

Analysis of concentration of molecular species after sonication by HPLC

It was not observed formation of insoluble agregates in the presence of all studied salts **(Figure 3).**

NaH_2PO_4 is the salt that provided more protection of protein (Dtxd): this salt increased monomer concentration and decreased fragment A and B concentration compared to the other studied salts **(Figure 3).**

KSCN increased the FA and FB (Dtxd peptides) contents It means: this salt induced protein damages **(Figure 3).**

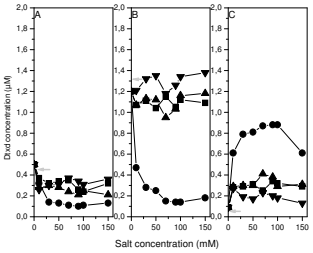

Figure 3. Effect of Hofmeister ion series salts in the Dtxd concentration of molecular species after sonication. The Dtxd samples were sonicated with Ethyl acetate ($CH_3CO_2C_2H_5$) in the presence of (-■-) NaCl, (-●-) KSCN, (-▲-) $MgCl_2$ e (-▼-) NaH_2PO_4. The samples containing 2 µM Dtxd were analyzed by HPLC gel filtration (flow = 0,6 mL/minute in PBS pH 7,2, 20°C). A. Dimmer concentration, B. Monomer concentration, C. Fragment A and B concentration. Control: not sonicated Dtxd in water (→).

Analysis by Fluorescence and Circular Dichroism

W intrinsic fluorescence: The tertiary Dtxd structure remained stable **(Figure 4).**

The Dtxd β-sheet content remained constant in the presence of all the studied salts **(Figure 5).**

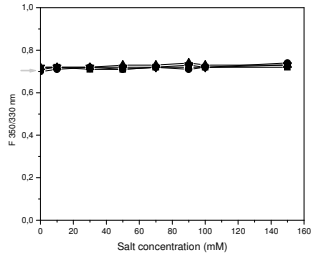

Figure 4. Relation between intrisic fluorescence at 350 and 330 nm of Dtxd samples in the aqueous phase after sonication. The samples (-■-) NaCl, (-●-) KSCN, (-▲-) $MgCl_2$ e (-▼-) NaH_2PO_4 after sonication with ethyl acetate ($CH_3CO_2C_2H_5$) were analyzed by flurescence espectroscopy. Control: not sonicated Dtxd in water (→).

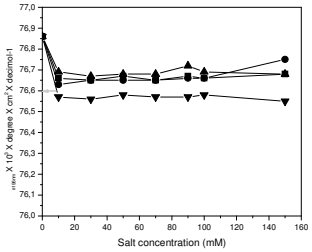

Figure 5. Effect of Hofmeister ion series salts in the Dtxd in the [θ]196 nm. The Dtxd samples (7,36 µM) in the presence of (-■-) NaCl, (-●-) KSCN, (-▲-) $MgCl_2$ e (-▼-) NaH_2PO_4, after sonication with ethyl acetate ($CH_3CO_2C_2H_5$), were analyzed by CD spectroscopy. Control: not sonicated Dtxd in water (→).

The Dtxd α-helix content remained constant in the presence of PO_4^{2-} **(Figure 6).**

The S-S dihedral angle remained stable in the presence of all studied salts **(Figure 7).**

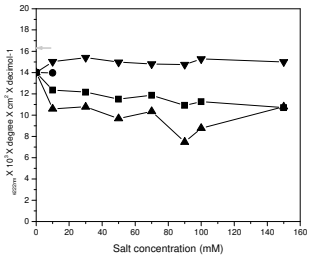

Figure 6. Effect of Hofmeister ion series salts in the Dtxd in the [θ]222 nm. The Dtxd samples (7,36 µM) in the presence of (-■-) NaCl, (-●-) KSCN, (-▲-) $MgCl_2$ e (-▼-) NaH_2PO_4, after sonication with ethyl acetate ($CH_3CO_2C_2H_5$), were analyzed by CD spectroscopy. Control: not sonicated Dtxd in water (→).

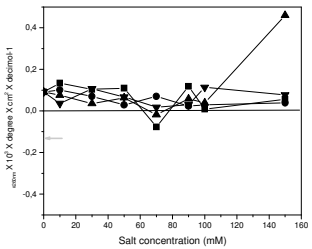

Figure 7. Effect of Hofmeister ion series salts in the Dtxd in the [θ]260 nm. The Dtxd samples (7,36 µM) in the presence of (-■-) NaCl, (-●-) KSCN, (-▲-) $MgCl_2$ e (-▼-) NaH_2PO_4, after sonication with ethyl acetate ($CH_3CO_2C_2H_5$), were analyzed by CD spectroscopy. Control: not sonicated Dtxd in water (→).

CONCLUSIONS

The Hofmeister salt series protected Dtxd during the REVS-Chi preparation. The Dtxd α-helical content was quite stable with minor exposition of hydrophobic residues (corroborated by measures on intrinsic tryptophan (Trp) fluorescence). REVS-Chi liberated soluble and immunological active Dtxd. The formulation can be use as vaccine particulate adjuvant.

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