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Methods of synthesis and biochemical properties of boron compound – protein conjugates

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Abstract

Boron-containing compounds are actually widely used as insecticides, bactericides, reducing reagents (organic chemistry), catalysts, materials for the production of some types of ceramics, the liquid crystal components, temperature-resistant polymers and many others. They have also been studied as potential drug. Modern chemical, biomedical and pharmaceutical sciences show a growing interest in unique type of boron compounds - boron clusters. Due to their structure, durability and exceptional properties under physiological conditions, these compounds appear to be excellent candidates for modifying peptides and proteins. Currently, the most commonly used in the biomedical research of boron clusters are compounds with icosahedral geometry with 12 peaks containing boron or boron and carbon atoms - carboranes. Both boron and carborane clusters have an extremely complex bond structure, where each of the boron or carbon atoms is associated with six other atoms. The electrons involved in structure formation are delocalized, which is why boron clusters have three-dimensional aromaticity. A single boron cluster is almost an ideal sphere only 50% larger than a rotating phenyl group. Compounds consisting of two or more carborane clusters co-ordinated with a metal atom - a metallocarboranes are equally common. One of the most stable clusters of this type are complexes with cobalt (Co), but also molecules with other metals are known, such as Fe, Cr, Nb, Ni, Cu, Au, Pt, Ru, Re or Tc. Boron clusters are abiotic substances with high durability, both chemical, thermal and biological. As chemical synthesis products, they do not have their equivalents in the nature known to us. As a result, active substances based on the compounds of boron clusters are removed from the body without interfering with their spatial structure.

The aim of this study is to evaluate the possibility of bioactive protein modification using boron clusters and to assess the impact of modifications on the physicochemical and biochemical properties of proteins. The tests were carried out using oxonium adducts (dioxane and tetrahydropyran) from 3 different boron clusters: closo-dodekaborban, dicarba-undekcarborane and bis (1,2-dicarbolido) cobalate. First, tests were carried out to assess the reactivity of the boron cluster adducts in the water-organic environment compared to the nucleophilic groups present in it. After selecting the appropriate reaction conditions, a series of reactions with amino acids and amino acids blocked on the alpha-amino group was carried out. These studies showed a very broad reactivity of the oxonium adducts against nucleophilic groups: -OH, -NH2 and -SH, as

well as arginine or histidine functional groups. On the basis of the conducted research, two procedures were developed for the synthesis of protein and peptide conjugates with a boron cluster: reaction in an aqueous-organic environment and solid phase reaction at elevated temperature. These two methods were used to synthesize conjugates of boron clusters with hen egg lysozyme. Fractions of mono-substituted conjugates were purified on hydrophobic interaction chromatography (HIC) and then analyzed by liquid chromatography coupled with mass spectrometry, circular dichroism and dynamic light scattering techniques. The combination of these techniques allowed to determine changes in the secondary, tertiary and quaternary structure of the protein resulting from the modification with boron clusters. The studies excluded the effect of conjugation with boron clusters on the secondary and tertiary structure of the protein. Modification caused significant changes in the behavior of conjugates leading to time- and temperature-dependent. aggregation At the same time, despite the decrease in the biological activity of conjugates, their aggregation compensated for the loss of lysis capacity of gram-positive bacterial cells. These studies have shown how a single molecule of the boron cluster can have a significant impact on the physical and biochemical properties of protein modification. Protein aggregation induced by conjugation with the boron group seems to be an undesirable phenomenon. In addition, parallel studies have demonstrated the ability of metalocarborane clusters to bind strongly to the hydrophobic cavities of human serum albumin (HSA). However, for many drugs, especially peptides, such multimerisation or binding to albumin are strategies that allow a change in pharmacokinetics, including an increase in the half-life of the drug system. In order to verify the possibility of transferring the binding properties of the metallocarborane cluster to albumin to the peptide molecule, a conjugate of boron conjugates with human insulin was performed. It has been shown that the fraction of mono-substituted metallocarbobate conjugates with human insulin has the ability to bind to HSA approximately 20-fold higher than the commercially available long-acting insulin detemir analogue. At the same time, the modification of amino acids responsible for the binding of insulin to the receptor was excluded, which could potentially be prevented.

Summing up the presented results, boron clusters can be perfect modulators of physicochemical properties of therapeutic proteins and peptides. Targeted in sites the active hydrophobic domains of the protein can increase the binding or receptor binding

strength of the substrate. Seemingly insignificant, from the point of view of affecting the structure of the first and second tertiary proteins, the modification significantly influences the interactions of low molecular weight ligands as well as macromolecules.