Poster

Combining SC-XRD, micro-PXRD and 3D ED in the structural description of dehydration of nucleotides and nucleotide-based drug crystals

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Nucleotides are one of the most important low-molecular-weight organic compounds in biochemistry. Their multi-functionality (nucleic acids biosynthesis, cellular energy transformations, signal transduction processes [1-3]) provided the basis for many of today's antiviral and anticancer drugs, which are their synthetic analogues [4,5]. These systems are still poorly understood from a structural perspective, especially from the point of view of a solid state. A characteristic feature of the vast majority of nucleotides and their derivatives is the water present in their crystals. It performs a number of functions, including mediating the formation of hydrogen bonds or filling the coordination sphere of metal ions. These hydrates are often sensitive to temperature and humidity conditions, which allows for controlled dehydration. Importantly, it surprisingly often follows the SC-to-SC (single crystal-to-single crystal) mechanism, which greatly facilitates further structural research. However, in many cases the single crystal becomes a powder due to water loss, which opens up not only the possibility, but sometimes the necessity of using other techniques such as micro-PXRD or 3D electron diffraction [6,7].

Here we present the first structural and dehydration description of one of the basic ribonucleotides, cytidine 5′-monophosphate in the acid form (CMP) and the antiviral drug tenofovir (synthetic analogue of adenosine 5′-monophosphate) in its biologically active form, using variable temperature SC-XRD, micro-PXRD and 3D ED techniques.

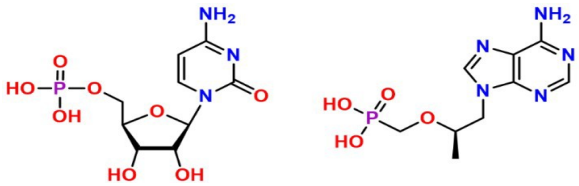

Figure 1. Chemical structures of cytidine 5′-monophosphate (left) and tenofovir (right).

Three varieties of CMP hydrates (CMP·xH$_2$O, x = 2, 1.5, 1) and a series of tenofovir hydrates (TEN·xH$_2$O, x = 5.5, 4.5, 3.8, 3.5, 3, 1) with different water contents were grown from solution and subjected to thermally induced and diffraction-controlled dehydration. A number of partially dehydrated and anhydrous forms were obtained. The crystal structures of one of the CMP hydrates and its anhydrous form, as well as tenofovir anhydrate (both dehydrated under vacuum conditions), were determined using electron diffraction. Individual forms with increasingly lower water content were structurally described and their formation process confirmed with VT-micro-PXRD measurements. These studies allowed not only to describe the water loss paths in these crystals, but also to identify the most important supramolecular motifs. It has been shown that from the crystal stability point of view, the most important are intermolecular interactions (hydrogen bonds and lone pair···π interactions) formed between phosphates/phosphonates and nucleobases (adenine, cytosine).

It was shown, that diffraction studies of nucleotide and nucleotide-based drug crystals subjected to dehydration may allow for a more complete understanding of their nature in the solid state and an attempt to hierarchize individual types of interactions, as well as a deeper description of the role of water in these systems.

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