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MOLECULAR MODELING AS A TOOL IN MOLECULAR BIOLOGY OF MEMBRANE-BOUND RECEPTORS

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The importance of computer modeling of membrane proteins in molecular biology is worked out. We give three examples that models gained by a combined approach of homology and energetic modeling with vibrational spectroscopy are a useful help in site-directed mutagenesis, truncation, binding-studies and even in crystallography. The study of the vanilloid receptor is a successful application of a computer model in the construction of truncations that served for the identification of functionally important protein parts. In the case of CD69 computer docking helped to identify the Ca^{2+}–binding site that was not observed in the crystal structure of this protein due to the non-physiological conditions of crystallisation.

for full paper see page

PROTON TRANSFER IN SHORT OLIGOPEPTIDES

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Peptides belong to key biomolecules. Their activity can be influenced by several impacts and the interaction with protons is one of them. Such a type of interaction can also influence behaviour of peptides in gas phase, which can lead to different way of fragmentation during mass spectroscopy analysis [1].

Our quantum-chemical study has been focused on detailed analysis of proton interaction with short oligopeptides. Density functional theory employing hybrid functional B3LYP and 6-31G(d',p') basis set was used. The study was performed on terminally blocked diglycine and triglycine models. It implies that the proton can only interact with the oxygen and nitrogen atoms of the amidic groups. Because of appropriate geometry, the proton transfer can occur between these positions [2, 3].

In general, the proton transfer process consists of two repeating steps. In the first step, the proton is moved around the double bond of the carbonyl group by isomerization from *E* to *Z* configuration. Then the process continues by proton jump between adjacent carbonyl oxygens. The isomerization processes have significantly higher activation barriers than the jump steps [3]. Also changes in proton transfer were examined when a single water molecule was presented in the system. Strong influence to all steps, and also active participation of the water molecule due to proton exchange processes was found.

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APPLICATION OF POWDER DIFFRACTION IN BIOLOGY? THE EGG-SHELL MICROSTRUCTURE

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In last years, renaissance of rather old and traditional technique - X-ray powder diffraction can be observed. This was initiated by both the interest in design of new materials (in materials science, physics and chemistry, where it plays the role of a basic method), and also by fast development in instrumental techniques - X-ray optics and detection which enhanced its possibilities.

Powder diffraction pattern contains different kind of information. Peak positions and intensities are related to crystal (atomic) structure, i.e. the type and size of lattice cell and atomic positions and consequently it can be used for structure refinement and even structure determination in some cases. As a finger print of each individual phase, the diffraction pattern can be an ideal tool for phase analysis.

However, there is much more hidden in the pattern. Variations of lattice parameters and intensitites can detect lattice defects. This is related to the so-called real structure of material, the term which is also used for structural features in the scale of nanometers, i.e. grains or subgrains. The topics which is now of great interest because of intense

research of nanomaterials. Powder diffraction analysis nowadays may include application of different diffraction geometries and analysis of peak positions, intensities and widths. This makes possible a complete PXRD analysis - phase analysis, structure refinement, stress, strain, crystallite size and texture analysis.

Can the technique be of any use for biologists? There have not been many applications yet. Main interest of biologists now seems to be directed to protein crystallography where even synchrotron single crystal diffraction may be insufficient. However, recently, an attempt to use powder diffraction for structure refinement of proteins has appeared [1, 2] too.

In present work, we have tried to perform more complete diffraction analysis of different egg-shells.

The biological function of the egg-shell is a chamber for embryonic development and from which the chick is able to emerge at the appropriate time. The requirements of the table egg industry are different. The industry sustains economic loss from cracked eggs and some of the cracking can be attributed to the deficiencies in the egg-shell structure. This is one of the reasons why the attention to eggshell is devoted [3-5].

The egg-shell consists of several mutually through-growing layers of $CaCO_3$. The innermost layer - mamilary layer (~100 μm) grows on the outer egg membrane and creates the base on which the palisade layer constitutes the thickest part (200 μm) of the egg-shell. The top layer is the vertical layer (5-8 μm) covered by the organic cuticle.

Different kinds of hen´s and bird´s egg-shells in the powder form or as a whole from both sides of the shell were examined by powder diffractometry and film back-reflection method. The powder patterns were evaluated by the fitting of diffraction profiles with the Pearson VII function. The lattice parameters, peak intensities and profile broadening were analysed. At the Bragg-Brentano setting (2 = 40) the Cu radiation penetrates approximately into the 9 μm of the egg-shell, so the measurements from the inner and outer shell surface can give evidence of the mamilary and palisade layer, respectively.

The results obtained on egg-shells of very different origins shown no significant differences in lattice parameters that correspond well to the PDF-2 values. The patterns contained only basic phase $CaCO_3$ (space group no. 167: R-3c) with a small addition of magnesium (0.3 wt. % , determined by atomic absorption). Diffraction patterns of powders obtained from all the eggs investigated correspond very well to the pattern of standard $CaCO_3$. The correspondence is very good including intensities. The patterns obtained from egg-shell powders are also very similar to the standard pattern, regardless larger line broadening.

However, there are differences between powders and both sides of the shells. For inner shell surfaces, the intensities are only slightly different than in powders (including standard one) but there is significant line broadening indicating fluctuations of lattice spacings (the mean local strain of about 0.2 %). On the other hand, for outer shell surfaces, there is much smaller broadening of lines, similar to powders, but significant changes of intensities indicating the 00l textures of grains. This is also an evidence of presence of two basic layers, structurally very different - mamilary and palisade. The meaning of crystallographic texture has been emphasized [3, 4]. It was steted that the breaking strength of the eggshell is inversely related to the degree of calcite orientation and conversely, reduced strength in the eggshell from aged hens coincides with a high variability of texture [3].

As a general conclusion and amazing fact, we can say that any differences of XRD parameters between the eggs of very different origin are not significant. So that their microstructure and composition, as they can be seen by XRD, are the same.

This work was an attempt for non-traditional application of powder diffraction and it was shown that it may be helpful for biologists not only for phase analysis but also for the study of nanostructure of inorganic crystalline phases in biological objects which is closely related to the overall microstructure which is strongly influenced by proteins taking part in the egg creation. The eggshell matrix proteins influences the process of crystal growth by controlling size, shape and orientation of calcite crystals. The formation of avian eggs belongs to most rapid mineralization processes known.

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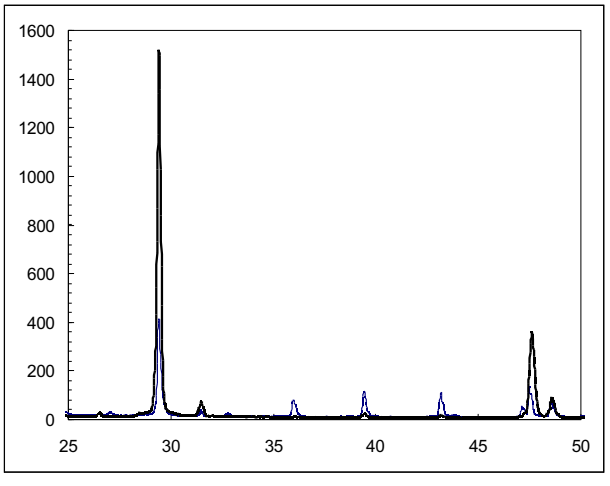

A typical part of the diffraction pattern of the egg-shell ($CaCO_3$) - from the inner (thin line) and outer side (thick line), respectively.