INTRODUCTION

Comparative characterization of the structure and morphology of hydroxyapatite crystals precipitated as a result of the chemical reaction between calcium and phosphate ions in aqueous solutions under various conditions is important for understanding the mechanisms of crystal growth, including biomineralization and optimization of technology of production of synthetic biomaterials. High-resolution electron microscopy (HREM) allows one to study material structures on the nanometer scale using fast Fourier transform (FFT) of images. The respective diffractograms (FFT) contain the information on the crystal lattice and sample orientation. Simulation of HRTEM images for the known crystal lattice and the zone axis allows one to determine the sample structure on the subnanometer scale and its dimensions along three directions, which, in turn, allows one to follow the mechanism of crystal growth.

The characteristic feature of hydroxyapatite (HAP) morphology is the pronounced shape anisotropy—thin crystalline plates are elongated in the [0001] direction. Two plate parameters (length and width) can readily be determined from the electron microscopy images, whereas the third parameter—crystal thickness—cannot be determined directly and accurately from micrographs. Thus, all attempts to determine the minimum dimension by orienting a crystal with its large face rigorously parallel to the beam have failed, because crystals in this position acquire a considerable charge, and crystal irradiation induces sample drift and deformation. Therefore, one has to determine the thickness on an “in-plane” oriented crystal. The use of thickness contours, stereopairs, and convergent-beam electron diffraction patterns [1, 2] in the thickness range of several nanometers (characteristic of precipitated hydroxyapatite crystals) is not possible either. Analysis of the intensities of X-ray energy-dispersion spectra [3] cannot help either, because of possible lattice distortion caused by radiation damage produced by a focused beam.

Thus, one of the most informative and least destructive methods of determination of the thickness of thin HAP crystals is HRTEM simulation and the subsequent comparison of the simulated images with the known experimental data for their best fitting at the set thickness of the object. Earlier [4–6], we applied this method for determining thickness in the identification of calcium phosphate crystals. However, simulation of some images or some regions of these images gave no satisfactory agreement with the experimental data because of the high sensitivity of crystals to irradiation with electrons, crystal bending, and block structure, which considerably hindered the rigorous orientation of the zone axis. All these difficulties required the introduction of new parameters in image simulation and, first of all, a parameter that would describe the deviation of the crystal orientation from the zone axis associated with possible grain misorientation or crystal bending. Thus, in addition to the three necessary parameters (crystal structure, sample thickness, and defocus), one has also to take into account crystal orientation (both its amplitude and azimuthal angle). For attaining better agreement between the calculations and experiment, we also
took into consideration a possible drift and vibrations of the sample. The correction for astigmatism was introduced directly in the process of electron microscopy investigation.

The present study demonstrates good possibilities of the modified simulation software for description of rather complicated situations arising in the interpretation of HRTEM images of large multiatomic unit cells with a large number of light atoms, such as Ca₁₀(OH)₃(PO₄)₆. The estimates of small thickness (one lattice parameter) of as-grown crystals and the subnanostructure determined show that attempts to establish the growth mechanism of thin crystals by studying their morphology and structure using HRTEM images are quite justified, as they allow one to work at a near-atomic level.

MATERIALS AND METHODS

Hydroxyapatite crystals were precipitated from dilute aqueous solutions as a result of the chemical reaction between calcium chloride and potassium dihydrogen phosphate at pH = 5.5–7.5 and various rates of mixing the initial solutions in the temperature range T = 25–95°C [4–6]. Precipitated crystals were washed in distilled water, dried, and transferred onto copper grids preliminarily coated with carbon films for further electron microscopy study. No additional thinning was used, because the crystals were sufficiently thin for electron diffraction analysis and obtaining high-quality HRTEM images.

Biosamples (mineral precipitates on cardiac valves) were prepared in the same way as the samples synthesized in aqueous solutions. The samples of bone tissues (bovine limbs and pieces of broken bones from various parts of the human spine) were washed in sodium chloride solution and dried. Then, the samples, about 2 × 2 × 2 mm in size, were filled up with epoxy resin so that it penetrated the sample pores. Using the method of ultramicrotomy and a diamond knife, we obtained thin (50–70 nm) slices of the solidified composite thus prepared. It should be noted that, to avoid possible artifacts in electron diffraction analysis and interpretation of HRTEM images, none of the indicated samples was stained or coated either with carbon or metal film.

The samples were studied on a Philips CM300UT FEG high-resolution electron microscope. The images were obtained with the aid of a CCD Gatan797 camera (1024 × 1024 pixel × 14 bit). The experimental images were quantitatively processed using the Digital Micrograph 3.6.1 computer program.

Electron diffraction patterns and HRTEM images were analyzed and interpreted based on their simulation using the Java Electron Microscopy Simulation (JEMS) software [7] for hexagonal hydroxyapatite crystals (sp. gr. P6₃/m) with the lattice parameters a = 0.942 nm and c = 0.688 nm [8].

JEMS SIMULATION OF HRTEM IMAGES

JEMS [7] is a multifunctional software designed for simulating HRTEM images (by the multislice and Bloch-wave methods), single-, polycrystal, and convergent-beam electron diffraction patterns (within the frameworks of both kinematical and dynamical theories), transfer functions for all the types of electron microscopes, and constructing crystallographic models of various substances in both direct and reciprocal spaces, stereographic projections, and three-dimensional images. In comparison with the earlier EMS simulation software [9], the recent version, written in the Java language for a new user’s graphic interface, allows one to vary any sample or image parameter at any moment. Setting the necessary parameters, it is possible to observe, e.g., the changes in HRTEM images in real time. An important advantage of JEMS is the high speed of computations and new possibilities for interpreting results associated with sample drift, vibrations, tilt, astigmatism, and image shift. In studies of the compositions of multiphase systems, JEMS allows one to analyze electron diffraction patterns and identify the constituent phases in a large number of compounds simultaneously. Thus, in order to confirm the presence of the hydroxyapatite phase, each electron diffraction pattern obtained was compared with 15 calculated diffraction patterns of different modifications of calcium phosphate.

We used JEMS to simulate electron diffraction patterns, HRTEM images, and their diffractograms. The procedure of simulation of HRTEM images consisted in the following. First, we input into the program the following parameters of the microscope used (e.g., of a Philips CM300UT FEG microscope):

—accelerating voltage 300 kV,
—spherical-aberration coefficient 0.65 mm,
—chromatic-aberration coefficient 1.2 mm,
—resolution at a 45-nm-Scherzer defocus of 0.17 nm,
—divergence of the electron beam (half-illumination angle) 0.8 mrad, and
—deviation from the focus distribution 4.0 nm.

The parameters for simulating images, namely, the initial defocus, the step in the defocus variation, the set of defocus values, the dimensions of the simulated images along the x and y directions, and the level of random noise were set directly in the process of simulation; the number of iterations determined the crystal thickness.

JEMS allows one to study the effect of the incident-beam tilt either by displacing the objective-lens aperture or by tilting the sample. In the present study, we assumed that the incident beam is normal to the sample surface and considered only the effect of the beam deviation from the zone axis. The crystal tilt in the JEMS program is described by positioning the Laue circle center (CLC) in the reference system of reflection indi-