Identification of H-Bonds in a Calcified Aortic Valve

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Abstract—The IR absorption spectra of subendothelial regions of the aortic valve cusps of a patient with calcified aortic stenosis diagnosis were measured by the attenuated total reflectance (ATR) spectroscopy technique in a frequency interval of $2500-3600$ cm^{-1} and analyzed in comparison to the spectra of healthy human tissues and reference spectra of a pig's healthy bone and aortic valve. The IR absorption band structure in this spectral region was studied by means of expansion into Gaussian components. The energies of hydrogen bonds (H-bonds) involving O−H groups were estimated. It is established that, among all samples studied, the energy of H-bonds between hydroxyl groups reaches maximum in the bone tissue.

As human life expectancy in developed countries has significantly increased, investigations devoted to the principles of pathogenesis of disorders inherent in aged and senile patients become more and more important. One of the most illustrative examples in this respect is offered by calcified aortic stenosis that is developing predominantly in patients above 70 years old and manifested by the calcification of aortic valve cusps and related disorders of hemodynamics.

The pathogenesis of calcific aortic stenosis is still not completely clear, and, hence, no any effective methods of prophylaxis have been proposed so far. This situation makes investigations devoted to elucidation of the principles of this mysterious disease topical because they can eventually lead to the development of effective methods for its prophylaxis and therapy. In the pathogenesis of diseases of this group, important role probably belongs to peculiarities of the formation of hydrogen bonds (H-bonds) in the appearing calcinates and between these calcinates and amino acid residues of proteins [1, 2]. Recently, we have formulated [2] a hypothesis concerning the nucleation of bone tissue clusters—hydroxyapatite—with the participation of H-bonds. It was shown with the aid of X-ray diffraction techniques [1] that hydroxyapatite nucleation proceeds in a porous space of subendothelial aortic structures to which the blood flow gets access due to the development of a pathological state of endothelial dysfunction.

In the present work, the method of IR spectroscopy with the attenuated total reflectance (ATR) technique has been used to confirm this hypothesis. A spe-cial feature of this technique is the possibility to measure IR absorption spectra in the subsurface region of tissue samples—that is, in the case of aortic valve demilunes—to gain information about short-range atomic vibrations in the sub-endothelial region.

As is known, H-bonds being the weakest contacts among intermolecular interactions are subject to reversible rupture and recovery under the action of thermal motions of molecules at temperatures characteristic of a living organism. In this Letter, we present the results of a comparative analysis of the structure of IR absorption bands for protein tissues of various origins, with emphasis on the so-called "Amide A combination band." The interest in this absorption band is determined primarily by its involvement in the lines of hydroxyl ($-$OH) and amine ($=$OH and $-$NH$_2$) groups participating in the formation of complexes with H-bonds.

While the influence of H-bonds on the vibrational frequencies of OH groups and their intensities has been rather thoroughly studied [3, 4], the properties of nitrogen-containing groups are still not known in sufficient detail. For this reason, the characteristics of H-bonds will be considered in more detail in respect of the former groups and estimations of their binding energies [4]. For this analysis, we have selected the samples of various tissues: (i1) healthy male aortic valve, (i2) fragment of aortic valve cusp of a patient with calcified aortic stenosis diagnosis, (i3) fragment of healthy bone tissue, and (i4) synthetic collagen with reference to a healthy pig's aortic valve tissues. The tissue samples from a patient with calcified aortic steno-
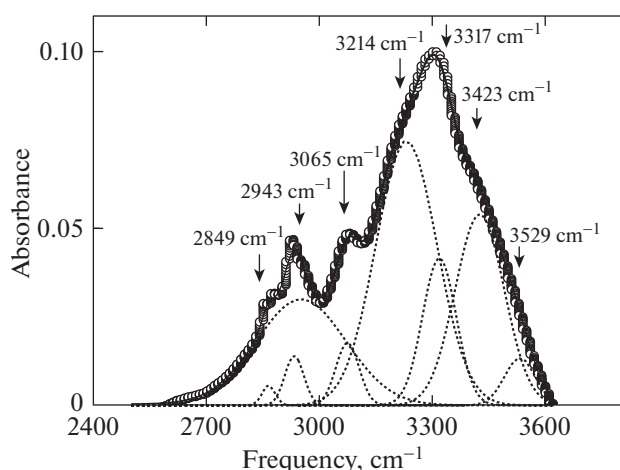
Fig. 1. Amide A band spectrum of a healthy human aortic valve tissue and its deconvolution to Gaussian components: (circles) experiment, (dotted curves) Gaussian component profiles, and (thick solid curve) envelope of the Gaussian profiles.

Fig. 2. Amide A band spectra of (*1*) aortic valve tissue damaged by calcified aortic stenosis, (*2*) healthy human bone tissue, and (*3*) healthy human aortic valve cusp.
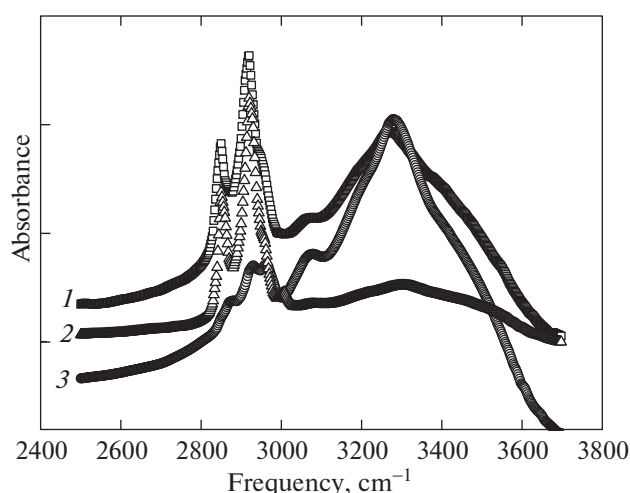
sis were taken upon surgical invasion for prosthetic aortic valve replacement.

The fragments of extracted calcified aortic valve demilunes were dried and the weight-average surface densities of calcified regions (expressed in Hounsfield units [5]) were multiplied by the total area of calcinates. The X-ray diffraction pattern typical of all studied samples of calcinates from average-severity patients (with Agatson index 143) was reported previously (see also [1]). The IR absorption spectra of nontransparent samples were measured using a single-reflection ATR sampling accessory with a ZnSe plate at 4-cm^{-1} resolution on a Nicolet 6700 Fourier spectrophotometer. Spectra were accumulated for 30−100 scans. The measurements were performed in the Geomodel Equipment Sharing Center at St. Petersburg University. The ATR-IR spectra are measured for a sample surface layer with thickness on the order of the probing light wavelength, so that the aortic tissue spectra correspond to its subendothelial regions.

Figure 1 presents a fragment of the IR absorption spectrum of a healthy human aortic valve tissue that provided a basis for the subsequent comparative analysis. The analysis was performed using the algorithm of least squares [6] for the best nonlinear fitting of

experimental curve to Gaussian component profiles. The result of a fitting procedure is shown in Fig. 1 by dotted Gaussian component profiles and their envelope (thick solid curve). As can be seen, successful fitting is ensured by a sum of eight Gaussian components, for which the root-mean-square deviation does not exceed 0.01%. The components with maxima at 2849 and 2943 cm^{-1} correspond to the well-known absorption bands of methyl−methylene groups, which are usually not involved in H-bond formation and, therefore, excluded from subsequent considerations. The band peaked at 3065 cm^{-1} corresponds to absorption on the valence vibrations of amino acid NH$_2$ groups, which (despite being involved in H-bonds) are also excluded from analysis for the aforementioned reasons. In contrast, the band at 3214 cm^{-1} can be assigned to the absorption on OH groups that are involved in H-bonding. Parameters of the Gaussian profile of this component are used for estimating the energy of H-bonds. Additional evidence for validity of the proposed interpretation is provided by the previously established assignment of the band at 3317 cm^{-1} to valence vibrations of the collagen NH group [7, 8] and the bands at 3423 and 3529 cm^{-1} to valence vibrations of the −OH group [8]. The absorption at 3317 cm^{-1} is typical of the NH groups of collagen [7, 8].

Now let us use the established frequencies of OH group vibrations for estimating the binding energies of complexes with H-bonds of the OH...A type using the shift of these frequencies from the frequency of valence vibrations of the OH groups free of H-bonding (3650 cm^{-1}) [3]. This estimation is based on the empirical correlation relationship between this frequency shift and the energy of H-bond formation [6]. The results of this analysis are presented in Table 1. As can be seen from these data, the healthy human aortic

Table 1. H-Bond formation energies in healthy human tissues

Vibrational frequency of OH groups, cm^{-1}	Frequency shift of OH groups, cm^{-1}	H-bond energy, kJ/mol
3214 ± 25	421	22.9
3423 ± 15	227	15.8
3529 ± 18	121	7.1
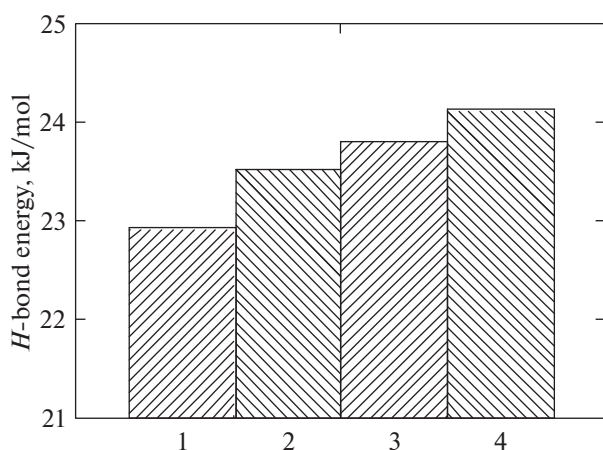
Fig. 3. Comparative diagram of H-bond energies in (*1*) healthy human aortic valve, (*2*) aortic valve damaged by stenosis, (*3*) synthetic collagen, and (*4*) bone tissue.

valve tissue has the H-bond energy close to that in water (20−25 kJ/mol [9]).

It is interesting to compare the data for the aortic valve tissue samples of a healthy human to analogous values for its bone tissue and to data for the aortic valve tissue damaged by calcified stenosis. This comparison is illustrated by the IR absorption spectra presented in Fig. 2. As can be seen, all the three spectra retain their general shape but differ significantly in respect of the mutual contributions to the absorption related to OH and NH_2 groups, on one hand, and the absorption on vibrations of methyl-methylene groups, on the other hand. A noteworthy close analogy is observed between spectra of the damaged aortic valve tissue and the bone. In addition, both these spectra are noticeably different from the spectrum of healthy human tissue. This difference can be interpreted as evidence of the fact that the damage of aortic valve proceeds by mechanism of the bone tissue elements (hydroxyapatite) nucleation in aortic subendothelial structures.

In order to pass from the qualitative characteristics of spectra in Fig. 2 to their quantitative analysis, let us employ the procedure used above for analysis of the shapes of spectral bands in Fig. 1. The results of estimation of the energies of H-bond formation are presented by a diagram in Fig. 3 in comparison to the

H-bond energy in dehydrated synthetic collagen. As can be seen from these data, the energy of H-bonds between hydroxyl groups reaches maximum in the bone tissue, where it even exceeds its value in synthetic collagen. This increase in the absolute value of energy corresponds to a decrease in the distance between interacting hydroxyl groups and, hence, to a greater density of the protein tissue.

Thus, the obtained results show evidence for the formation of bone tissue elements in subendothelial structures of aortic valve demilunes in patients with calcified aortic stenosis diagnosis. Another conclusion is concerning the significance and applicability of the proposed analytical techniques.

COMPLIANCE WITH ETHICAL STANDARDS

This work did not include experiments involving human beings or animals as objects of investigation.

CONFLICT OF INTEREST

The authors declare that they have no conflicts, of interest.

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