NEW BIOMEDICAL TECHNOLOGIES

Some Biochemical Characteristics and Water Exchange in Human Articular Cartilage in Osteoarthrosis

S. S. Nikolaeva, A. A. Roshchina, Kim Zon Chkhol, V. A. Bykov, G. A. Rebrova, O. A. Koroleva, L. V. Yakovleva, Yu. V. Abramov, and L. B. Rebrov

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Rearrangement of intra- and intermolecular bonds in collagen molecule, disaggregation of proteoglycans and their elimination from cartilage involved in osteoarthrosis are responsible for water accumulation and its increased mobility in cartilage.

Key Words: *cartilage; osteoarthrosis; collagen; proteoglycans; humor*

Osteoarthrosis is a highly prevalent articular diseases [3,10]. Despite numerous biochemical, physicochemical, and morphological studies [8,9,11], some aspects of the pathogenesis and progression of this disease are little studied, for example, structural changes in the basic matrix components of the cartilage and their correlation with water exchange in this tissue. We studied changes in biochemical and water exchange characteristics of human articular cartilage in osteoarthrosis.

MATERIALS AND METHODS

Articular cartilages of human femoral head were obtained from patients with osteoarthrosis (during surgery) or from subjects died from traumas (no later than 24 h postmortem). Non-aggregated proteoglycans were extracted with sodium chloride. Aggregated proteoglycans were extracted with 4 M guanidine chloride in the presence of protease inhibitors [12]. After centrifugation and dialysis, the extracts were analyzed by gel filtration on a Toypearl HW-75 column [1]. Enzymatic proteolysis of collagen in the cartilage with collagenase, pronase, and pepsin was carried out.

The content of hydroxyproline [14], hexosamines [4], and uronic acids [7] were measured, glycosylation of insoluble collagen was analyzed by the intensity of fluorescence of alkaline hydrolysates of cartilage tissue; the molecular weight distribution of hydrolysis products was evaluated by filtration on a Toypearl HW-55 column [5]. Hydratation of the cartilage tissue was studied using aquametric methods: titration with Fisher reagent, thermal analysis, and adsorption method [2].

RESULTS

The content of hexosamines and uronic acids in osteoarthrosis decreased 1.5 and 1.2 times, respectively (Table 1), while the content of collagen remained practically unchanged. Treatment with guanidine chloride removed about 56% hexosamines and 36% uronic acids from the control cartilage and 73 and 40% from osteoarthritic cartilage, respectively. These data attest to weakened bonds between collagen and proteoglycan in osteoarthrosis.

The total water content and the fraction of free water are increased in osteoarthrosis, while the frac-

VILAR Research, Training, and Methodological Center of Biomedical Technologies, Moscow

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TABLE 1. Content of Water and Glycosaminoglycans in Articular Cartilage of the Femoral Head

Parameter	Control	Guanidine chloride	Osteoarthrosis	
			without treatment	+guanidine chloride
Water, g/100 g tissue				
total	70.5±0.5	77.5±0.4	80.90±0.5	—
bound	15.4±0.2	11.0±0.2	1.77±0.1	—
free	55.1±0.5	65.5±0.4	79.1±0.4	—
α_{max}	37.9±1.7	39.0±0.5	47.1±0.5	—
Hexosamines, g/100 g dry tissue	5.05±0.28	2.23±0.29	3.40±0.56	0.93±0.03
Uronic acids, g/100 g dry tissue	4.23±0.45	2.73±0.13	3.47±0.26	2.07±0.12

tion of bound water decreased 9-fold (Table 1). Thus, the cartilage involved in osteoarthrosis contains primarily unbound water. Simultaneously, water-adsorption capacity of the cartilage at maximum relative humidity (α_{max}) also increased. Similar increase in the total water content and redistribution of water to the unbound fraction was observed after *in vitro* removal of glycosaminoglycanes from the cartilage.

For elucidation of the mechanisms underlying accumulation and redistribution of water in the articular cartilage in osteoarthrosis we studied biochemical characteristics of the main components of the articular

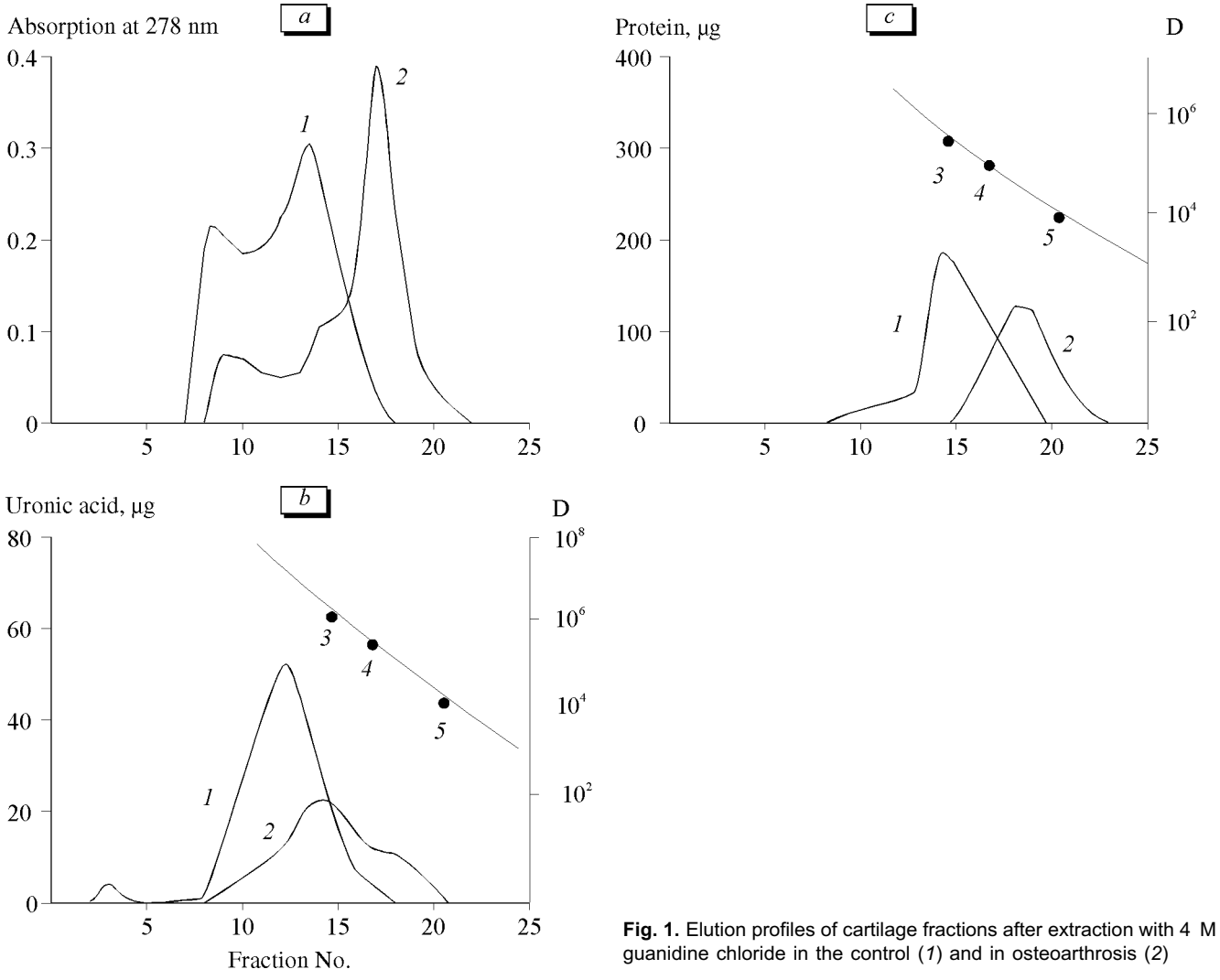

Fig. 1. Elution profiles of cartilage fractions after extraction with 4 M guanidine chloride in the control (*1*) and in osteoarthrosis (*2*)

cartilage matrix in this pathology. We found that molecular weight of carbohydrate and protein components was decreased in pathological cartilage preparation (Fig. 1). Cartilage involved in osteoarthrosis was characterized by a sharp decrease of peaks and predominance of low-molecular-weight components. These findings suggest that osteoarthrosis is associated with pronounced disaggregation of proteoglycans in the articular cartilage, disintegration of proteoglycans into monomers with subsequent degradation and depolymerization of the carbohydrate and protein components.

The loss of proteoglycans after treatment of the cartilage with guanidine chloride surpassed that in osteoarthrosis, but the increase in total water content and its redistribution in osteoarthritic cartilage were more pronounced than after *in vitro* removal of glycosaminoglycans. These findings suggest that the state of water and its bonds with biopolymers are determined not only by glycosaminoglycans, which are the most hydrophilic, but also by other components of the cartilage. The resistance of collagen in pathological cartilage to proteolysis decreased in comparison with the control (Fig. 2). The release of hydroxyproline-containing substances after treatment with all studied enzymes (pepsin, collagen, and pronase) increased. Treatment of pathological cartilage with specific enzyme collagenase produced a higher yield of low-molecular-weight products compared to normal cartilage. It is established that pepsin and pronase cleave collagen telopeptides, thus destroying intramolecular bonds. The weaker are these terminal sites of triple-stranded collagen molecule, the more it is vulnerable to proteolysis [6]. Unlike pepsin and pronase, collagenase cleaves helical regions of native collagen [13]. Decreased resistance of collagen in osteoarthritic cartilage to collagenase attests to weakening of intermolecular bonds in helical regions of the collagen molecules. This was confirmed by gel filtration of cartilage collagen in health and disease (Fig. 2).

Structural resistance of matrix collagen in pathological cartilage was also evaluated by the degree of

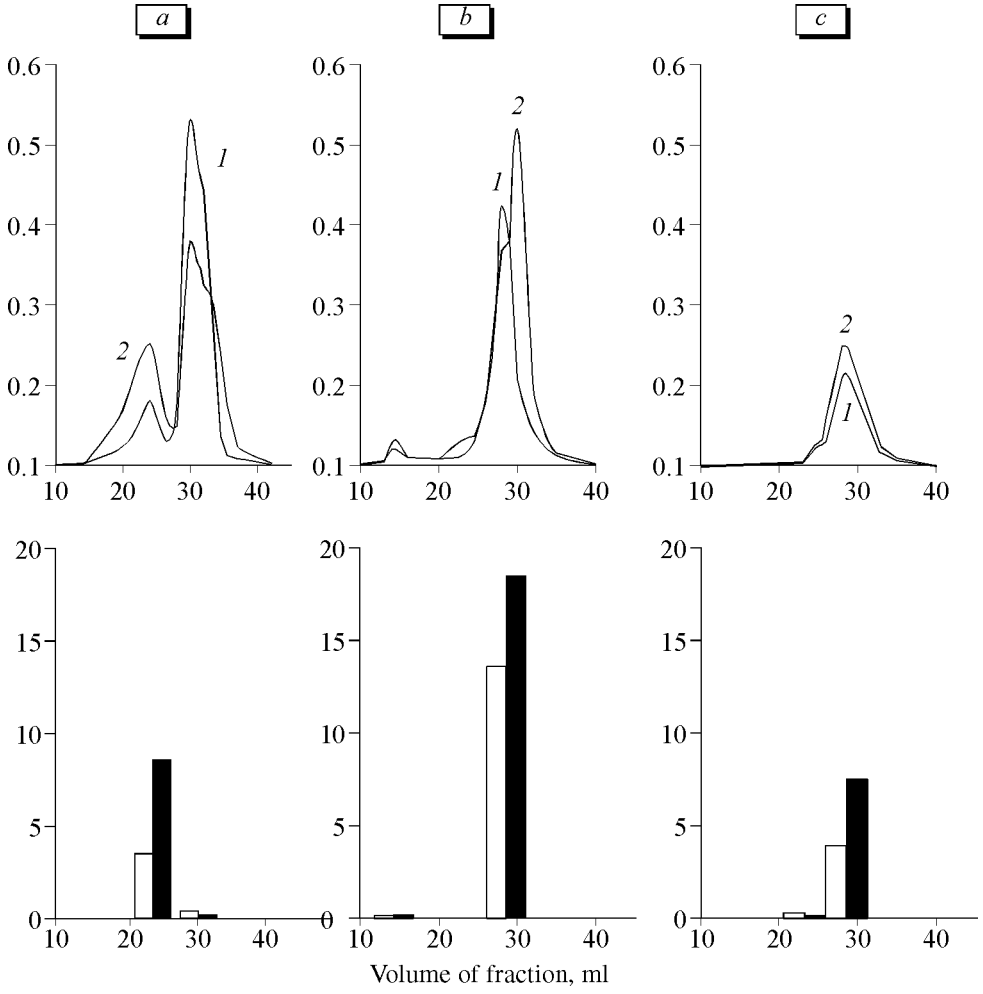

Fig. 2. Gel filtration of collagen proteolysis products in the control (*1*, light bars) and in osteoarthrosis (*2*, dark bars). *a*) pepsin; *b*) collagenase; *c*) pronase. Ordinates: E_{215} (top panels) and hydroxyproline content, μg (bottom panels).

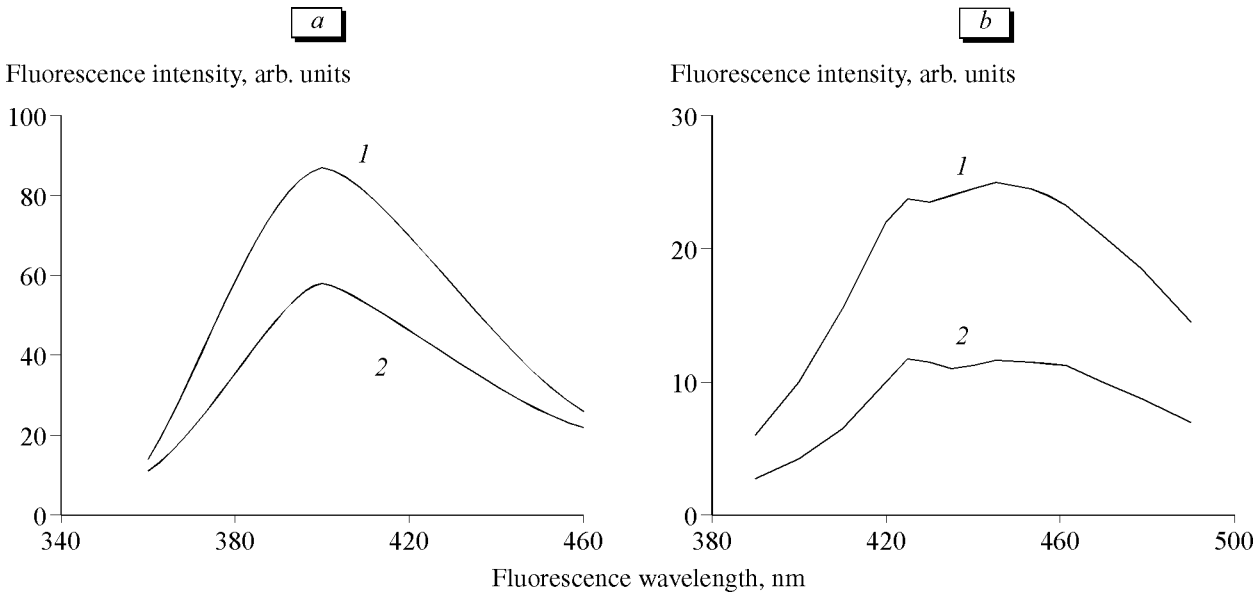

Fig. 3. Fluorescence spectra of alkaline hydrolysates of insoluble collagen fraction in normal (*1*) and in osteoarthrosis (*2*). *a*) excitation at 335 nm; *b*) excitation at 370 nm.

its glycosylation. The content of pentosidine in collagen hydrolysates of osteoarthritic and control cartilage differed significantly. The maximum of fluorescence spectrum (excitation at 370 nm, emission at 440 nm) of alkaline hydrolysate of insoluble collagen fraction from cartilage involved in osteoarthrosis was 2-fold lower than in the control (Fig. 3). Hence, cartilage collagen in osteoarthrosis is characterized by lower degree of glycosylation and lower content of chromophore-containing compounds, which participate in the formation of extra-, intra-, and intermolecular bonds in collagen molecules.

Hence, weakened bonds between the main matrix components in the cartilage and decreased content and disorganization of proteoglycans in osteoarthrosis, paralleled by a decrease in intra- and intermolecular bonds in collagen molecules are responsible for increased content of water and its increased mobility, which impair amortization characteristics of the cartilage matrix in osteoarthrosis. Bound water is responsible for additional intra- and intermolecular stabilization of collagen structure. Decrease of bound water fraction can be one of the causes of decreased stability of collagen molecule and subsequent destruction of the cartilage matrix collagen network fibers in osteoarthrosis.

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