Associating Hyaluronan Derivatives: A Novel Horizon in Viscosupplementation of Osteoarthritic Joints

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The association of two high-molecular-weight hyaluronan (HA) derivatives, namely a β-cyclodextrin (HA–β-CD) and an *N*-acylurea (EDC–HA), dissolved in aqueous NaCl, was studied. The weight-average of the molecular weights (M_w) of HA–β-CD and of EDC–HA was 185.3 and 86.8 kDa, respectively. However, the M_w value determined for the equimolar mixture of the two biopolymers equaled 556.0 kDa. Similarly, the radius of gyration (dimension) $Rg = 80.6$ nm of the above equimolar mixture was significantly greater than the values found for the single macromolecules, *i.e.*, 40.2 nm for HA–β-CD and 23.8 nm for EDC–HA. These data indicate that the two kinds of substituents, borne by the HA polymeric chains, form host–guest inclusion complexes resulting in polymacromolecular associates/aggregates.

Introduction. – Arthritis belongs among the most common afflictions of mankind. Of the more than 100 different types of arthritis, the most frequent is osteoarthritis (OA), one of the more expensive and debilitating diseases [1]. In OA, the synovial fluid (SF) is more abundant and less viscous: the concentration of hyaluronan (HA) is decreased, and the HA molecular weight is reduced [2]. These changes have been postulated to be co-responsible for the subsequent accelerated destruction of the cartilage [3]. Thus, logically, intra-articular HA-injection therapy termed 'viscosupplementation' has been implemented in the treatment of traumatized arthritic joints. However, the time during which intra-articularly deposited hyaluronan can exert its effect on the joint tissue is short. The half-life of HA following its injection into the joint (rabbit knee) is *ca.* 13 h [4]. The turnover of endogenous hyaluronan in human joints does not extend much over 0.5–2 days [5].

In the United States, two HA preparations, *Hyalgan*® and *Synvisc*® *G-F 20*, have been approved for intra-articular injection in symptomatic patients with OA of the knee [1]. *Hyalgan*® is an ultrapure high-molecular-weight hyaluronan with the molecular weight of *ca.* 5×10^5 Da on the average. *Synvisc*® *G-F 20* along with high-molecular-weight hyaluronan contains *Hylan*®, a gel prepared *via* HA cross-linking with formaldehyde [6]. As reported in [7], in the environment of an inflamed joint, where free radical species may be released, *Hylan*® gel retains its integrity better than the non-crosslinked hyaluronan macromolecules themselves.

To solve the task of effective viscosupplementation, a novel approach has, however, been suggested [8]. This approach comprises an intra-articular injection of two associating high-molecular-weight HA derivatives along with an appropriate low-molecular-weight drug competitor. On injecting such a 'cocktail', the drug molecules

initially should completely block the process of association. The very complexation of macromolecular components *in situ* will take place only after the drug has been cleared from the articular space. The two macromolecular components are: *1*) hyaluronan whose polymeric backbone bears a host, *e.g.*, the β-CD oligosaccharide, and *2*) hyaluronan substituted with an appropriate organic guest moiety (*cf. Fig. 1*).

This paper presents experimental results of a pilot study supporting the above described phenomena. The association of the two solutes, HA–β-CD and EDC–HA, in aqueous NaCl was followed by application of the multi-angle light-scattering (MALS) technique.

Results and Discussion. – Inclusion complexation between a host (H) and a guest (G) molecule is governed by the following relationship: $H + G \rightleftharpoons HG$. On assuming a 1:1 HG complex, at thermodynamic equilibrium, the complex-stability constant, K, can be expressed as $K = [HG]/([H][G])$. Thus, for an equimolar mixture ($[H] = [G] =$ 1 mol/l), the molar concentration of the formed HG complex is governed only by the numerical value of the K parameter.

The log K values of the β-CD complexation with organic guest molecules ($\leq$ *ca.* 5 [9]) indicate the formation of weak, reversible complexes. However, the multiple pinning-up of the HA–β-CD with the HA–guest derivative should result in the formation of stable polymacromolecular associates/aggregates, an illustrative example of which is depicted in *Fig. 1*.

The main molecular-weight parameters of the HA–β-CD and EDC–HA derivatives as well as of their 1:4 and 1:1 mixtures are listed in *Table 1*. From a quantitative point of view, the values of M_w and Rg determined for the associate formed, especially in the 1:1 mixture of the two biopolymers, exceed significantly the parameters observed on measuring each individual macromolecular component.

Table. *Molecular-Weight Parameters of the Biopolymers Studied*

Parameter	HA–β-CD	EDC–HA	[HA–β-CD]/[EDC–HA]	
			1:4	1:1
M_w [kDa]	185.3	86.8	293.0	556.0
Rg [nm]	40.2	23.8	69.6	80.6

Fig. 2 shows the intensity of the scattered light determined for EDC–HA (curve *a*) and for HA–β-CD (curve *b*), as well as for their mixture at equimolar concentration. As evident, there is a great difference between the MALS signal determined experimentally and the one calculated on assuming the absence of association (curves *d* and *c*, resp.). The experimental MALS signal (curve *d*) corroborates the following statements: *i*) the resulting solution contains not a simple mixture but rather an associate of the two HA derivatives; *ii*) in the mixture, the angular variation of the scattered light is indicative of the large size of the polymacromolecular associates/aggregates formed.

The most promising application sphere of the engineered novel HA-based biomaterials lies in health-care promotion. The two presented derivatives, HA–β-CD and EDC–HA, may claim priority as the first pair of (self-)associating/aggregating

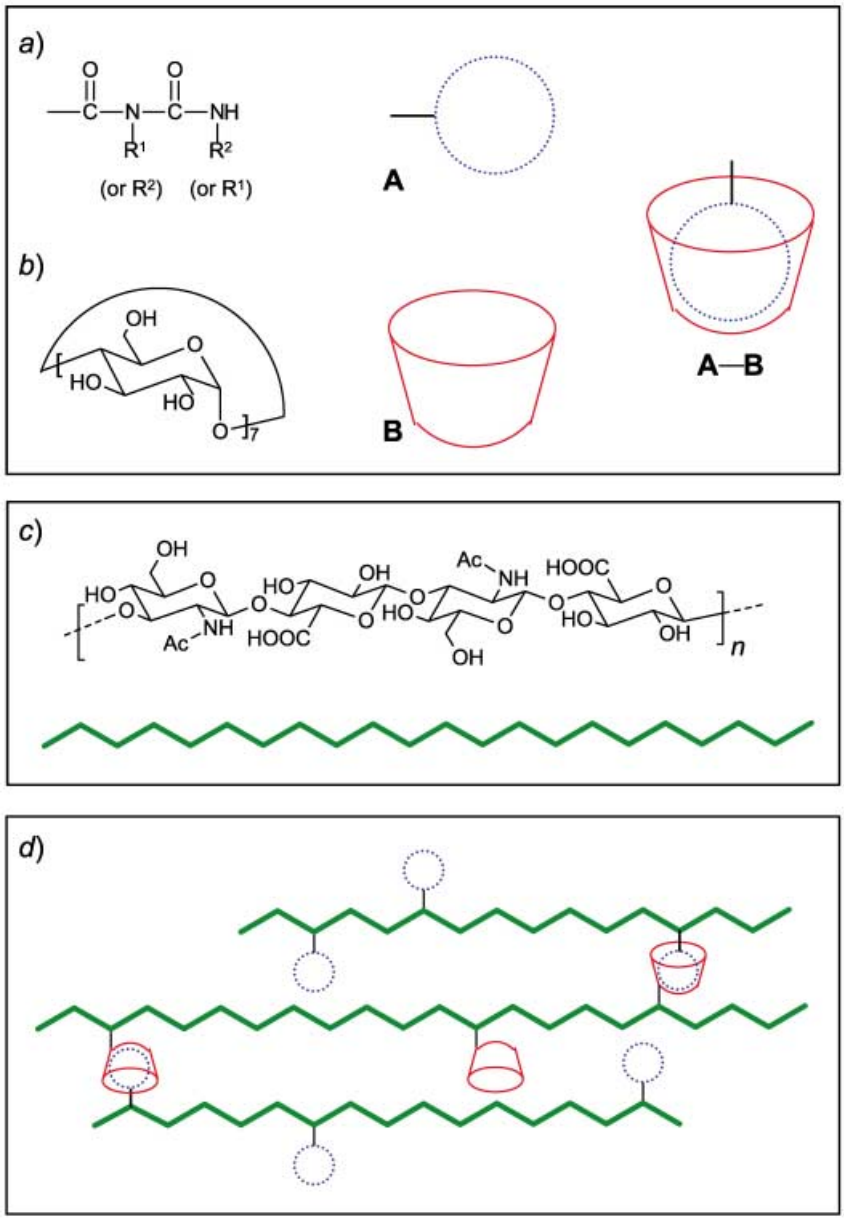

Fig. 1. *Chemical formulae of* a) N-*acylurea* ($R^1 = Et$, $R^2 = Me_2N-(CH_2)_3$) *and of* b) *β-cyclodextrin*, *as well as sketches of their higher-order structures* (**A** and **B**), *and of their inclusion complex* (**A**–**B**); c) *chemical formula of a high-molecular-weight hyaluronan and a sketch of its polymeric chain*; d) *sketch of the nHA–β-CD × mEDC–HA associates/aggregates*

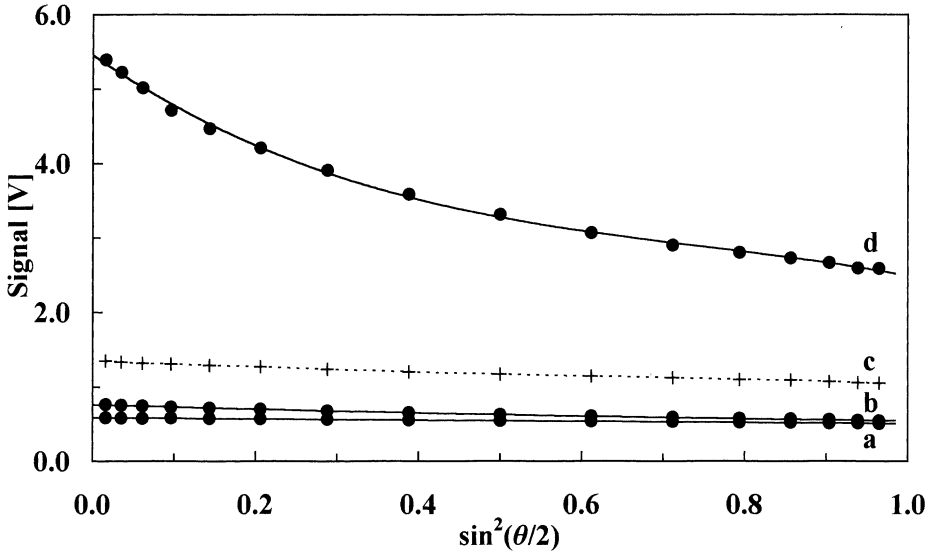

Fig. 2. *Intensities of the light scattered by the macromolecules of the two individual HA derivatives,* i.e., a) *EDC-HA and* b) *HA–β-CD as well as* c) *by the nHA–β-CD × mEDC–HA associates/aggregates measured or* d) *calculated on assuming a simple 1 + 1 additivity*

supramolecular HA biopolymers, opening up new horizons in a range of biomedical areas. Recently, similar experimental studies were presented [10].

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Experimental Part

HA Derivatives. The synthesis as well as the anal. protocol for the two HA derivatives, HA–β-CD and EDC–HA, was already described in detail in [11].

Multi-Angle Light Scattering. The light-scattering measurements (batch mode) were carried out at r.t. with a MALS photometer (*Dawn DSP-F*; *WYATT Technology*; Santa Barbara, CA, USA) equipped with a vertically polarized He-Ne laser (wavelength 632.8 nm). The angular distribution of the intensities of the scattered light was monitored by an array of photodiodes. The calibration constant for transforming the photodiode output voltage into the *Rayleigh* factor, $R(\theta)$, was determined with toluene as a standard ($R(\theta)_{\text{toluene}} = 1.406 \times 10^{-5}\,\text{cm}^{-1}$). The normalization of the photodiodes was carried out by measuring the intensity of the light scattered by a conc. soln. of bovine serum albumin (*Sigma*; *A 7888*), a globular protein assumed to act as an isotropic scatterer. The sample investigated was swollen-dissolved overnight in aq. NaCl (0.15M). Before the measurements, each sample soln. was filtered through an 0.2-μm cellulose acetate filter (*MILLIPORE Corporation*, Bedford, MA, USA). The MALS data-acquisition and analysis software used was *ASTRA 4.50* (*WYATT* Technology).

The specific refractive index increment, *dn*/*dc*, of the sample soln. was determined by a *KMX-16* differential refractometer (*LDC Milton Roy*; Rochester, NY, USA). The *dn*/*dc* value found (0.150–0.151 $\text{ml} \cdot \text{g}^{-1}$) was fairly identical for each sample soln. investigated.

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