Self-assembling of alkyl chains in acylated hyaluronan

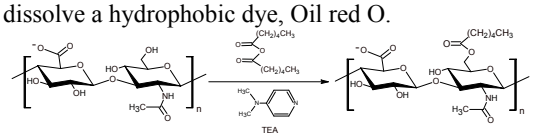
D. Šmejkalová, M. Hermannová, R. Šuláková, L. Palek, V. Velebný
Contipro C, Dolní Dobrouč 401, 561 02 Dolní Dobrouč.

Statement of Purpose: In the past few decades, intensive research has been directed towards a development of amphiphilic-based carriers that may serve as drug delivery systems. The bioavalability of these carriers is imparted by biopolymers forming either the interior or exterior network. Typically, these biopolymers are carbohydrates of natural origin, which are fully biocompatible and biodegradable. One of such a biopolymer is hyaluronan (HA). Due to its properties, mainly specific viscoelasticity, hydration and lubrication, a great attention is focused on HA in cosmetic and pharmaceutical industry. However, a suitable chemical modification of HA is required in order to increase the resistance of HA against enzymatic degradation and at the same time to introduce hydrophobic moieties into highly hydrophilic HA structure. One of such modifications may involve esterification of HA with fatty acids, ideally forming self-assembling carrier systems. Since the choice of reaction solvent may affect substitution position on HA chain, esterification of HA in polar protic and aprotic environments is expected to yield surfactant-like compounds with different structural and conformational properties.

Methods: HA sodium salt (HA-Na) and HA in its acid form (HA-H) were acylated (Ac) according to Scheme 1 in either DMSO/H_2O mixture (Ac-HA-Na) or solely DMSO solvent (Ac-HA-H), respectively.

Structural analyses were carried out by NMR (Bruker Avance III 500 MHz) and ESI-MS/MS technique (Maldi Synapt HDMS system Waters).

Self assembling of acyl chains into hydrophobic domains was examined by comparing the ability of HA derivates to dissolve a hydrophobic dye, Oil red O.



Scheme 1.

Results: ^{1}H NMR spectra of Ac-HA-H and Ac-HA-Na are reported in Fig. 1. Except for typical proton chemical shifts of HA involving a singlet at 2.0 ppm belonging to $COCH_3$ group, skeletal signals at 3.4-3.9 and anomeric resonances at 4.4-4.6 ppm, CH_2 resonances coming from acyl chain were detected at 0.8, 1.2, 1.5 and 2.4 ppm(a-e, Fig.1). The degree of substitution was determined to be 70% for both acylated products. Based on COSY, and TOCSY the upfield chemical shift of one of the HA skeletal signals in Ac-HA-Na detected at 3.1 ppm was attributed to H2 of glucuronic acid bonded with N-acetylglucosamine modified in position 4. No such observation was made in case of Ac-HA-H. HSQC spectra detected in both acylated derivates a downfield chemical shift of C6 and H6, specific for esterification of CH_2OH group of N-acetylglucosamine as shown in Scheme 1.This observation was further supported by MS data, clearly confirming the presence of one acyl chain per HA dimer, and hardly any acylation on glucuronic acid in Ac-HA-Na.

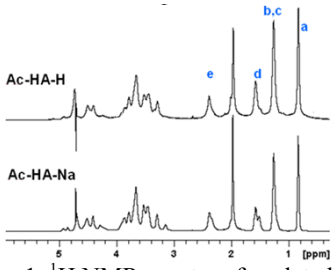


Figure 1. ^{1}H NMR spectra of acylated HA.

The absorbance of Oil red O bound by Ac-HA-H and Ac-HA-Na is reported in Fig. 2. Although both derivates had *Mw* around 50 kDa, the measured absorbance was much higher than that observed in unmodified HA. At certain concentration, Ac-HA-H was found to be behaving differently from Ac-HA-Na. In fact, these results suggested the presence of larger hydrophobic domains in Ac-HA-Na at higher concentration than 10 mgml^{-1}. This phenomenon may be explained by the vicinity of acyl chains within Ac-HA-Na secondary structure and easier formation of 'micelle-like' conformation as compared to Ac-HA-H. This suggestion was further supported by DOSY indicating a significant aggregation of acyl chains in Ac-HA-Na (Fig. 3).

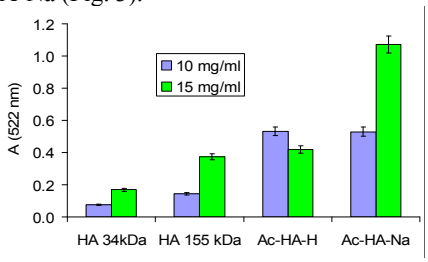


Figure 2. Absorbance of Oil red O.

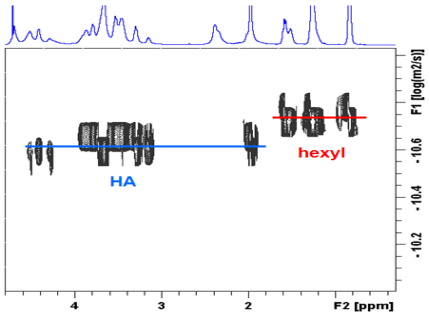


Figure 3. DOSY NMR spectrum of Ac-HA-Na (c=15 mgml^{-1}).

Conclusions: Acylation of HA in DMSO is more selective than that in DMSO/H_2O. However, esterification with lower regioselectivity leads to a more sterically feasible conformation supporting aggregation among acyl chains. Therefore, in case of lipid-based carrier HA systems acylation in polar protic solvent may form a more efficient carrier system.