

Glycobiology 2017: The interaction between dermal type I collagen and hyaluran (HA) in phosphate buffered solution, pH = 7.4.- Julian M Menter - Morehouse School of Medicine

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Abstract

As dermal collagen fluorescence spectra are time – and environment – sensitive, they show promise as biomarkers and prognosticators of damage due to aging and other pathology in general. The rate of photochemical formation of dityrosine from internal tyrosine residues is quasi – linear, and its slope can serve as an indicator of the rate of ground and excited state molecular damage. In Vivo dermal collagen is embedded in surrounding extra cellular matrix (ECM) containing a complex of hyaluronic acid (HA) and proteoglycan (PG). In this work, we report preliminary results of Collagen – HA interactions in an model in vitro system. Methodology: Solutions containing 1.0 mg/ml type I collagen + 2.0 mg/ml HA (Elastin Products) in 0.1 M phosphate buffer, pH 7.4 were irradiated from 0 – 200 min. in a thermostatted cuvette (Hellma Cells) with a 4 W filtered UVG – 11 hand lamp emitting at 254 nm. Dityrosine formation as a function of time was monitored by its fluorescence at excitation/emission wavelengths 325/400 nm for temperatures between 13 – 60 oC. Results and Discussion: For $T < T_m$ (~ 36 oC) HA retards the rate of dityrosine formation by ~ 20 – 30 %, indicating stabilization of collagen scaffolding by HA. At $T > T_m$, where the coiled conformation dominates, there appears to be no systematic effect of HA on collagen stability. Thus, stabilization of collagen helical structure seems to be one important function of the ECM.

Statement of the Problem: As dermal collagen fluorescence spectra are time- and environment-sensitive, they show promise as biomarkers and prognosticators of damage due to aging and other pathology in general. The rate of photochemical formation of dityrosine from internal tyrosine residues is quasi-linear and its slope can serve as an indicator of the rate of ground and excited state molecular damage. In vivo dermal collagen is embedded in surrounding extra cellular matrix (ECM) containing a complex of hyaluronic acid (HA) and proteoglycan (PG). In this work, we report preliminary results of collagen- HA interactions in model in vitro system. Methodology: Solutions containing 1.0 mg/ml type I collagen \pm 2.0 mg/ml HA (elastin products) in 0.1 M phosphate buffer, pH 7.4 were irradiated from 0-200 min in a thermostated cuvette (Hellma Cells) with a 4 W filtered UVG-11 hand lamp emitting at 254 nm. Dityrosine formation as a function of time was monitored by its fluorescence at excitation/emission wavelengths 325/400 nm for temperatures between 13-60oC. Results & Discussion: For $T < T_m$, where the coiled conformation dominates, there appears to be no systematic effect of HA on

collagen stability. Thus, stabilization of collagen helical structure seems to be one important function of the ECM.

Introduction: The extracellular matrix in mammalian dermis (ECM) is the non-cellular component, providing not only essential physical scaffolding for the cellular constituents and initiating crucial biochemical and biomechanical cues. In mammalian dermis, the predominant molecules are 1) Type I and Type III (85:15) collagens and 2) modular proteoglycans (PGs) Although many different types of molecular interactions are possible, they depend on the cells condition (i.e. “Normal”, Aged, Wounded/Fibrotic, and Cancerous). Furthermore, PGs themselves appear to bind to many cell-surface receptors with high specificity, thereby activating signaling pathways that control cell proliferation, differentiation, adhesion, and migration.

Previous studies document that collagen and HA interact with each other in the ground state under physiologically relevant conditions in rabbit synovium, in vitro and in aqueous solution . Results of rotary shadowing electron microscopy and computer simulation indicate that HA self-aggregates into highly-branched networks that can form two-fold aggregation structures at the ends of the helix. HA mixed with collagen in situ causes a shift in distribution of fibrils to smaller diameters . Hyaluronic acid (hyaluronan, HA) does not bind covalently to collagen, but collagen and HA do interact by mutual steric exclusion. This noncovalent interaction enables HA to form non-ionic complexes .

As the presence of dityrosine is diagnostic for protein damage, its presence in proteins has been proposed as a molecular probe of UV-induced photodimerization (reviewed in . There is little or no data that addresses the effect of the surrounding ECM on the photochemical production of dityrosine via excited state tyrosine (A*).

We have used a model in vitro buffered collagen/HA system to study the influence of HA molecules on UV-induced photodimerization. Shimazu found that the rate of photodimerization of tyrosine to dityrosine in aqueous solution at small irradiation times is quasi-linear and proportional to the initial tyrosine concentration [Ao]. Our preliminary results with collagen and HA reveal a complicated temperature dependence involving several factors relating to structure and conformation of the collagen, the HA, as well as photochemical activation parameters.

Theoretical: As dermal collagen fluorescence spectra are time – and condition – touchy, they show guarantee as biomarkers and prognosticators of harm because of maturing and other pathology all in all. The pace of photochemical arrangement of dityrosine structure inside tyrosine deposits is semi – straight, and its slant can fill in as a marker of the pace of ground and energized state sub-atomic harm. In Vivo dermal collagen is inserted in encompassing extra cell network (ECM) containing a complex of hyaluronic corrosive (HA) and proteoglycan (PG). In this work, we report fundamental consequences of Collagen – HA collaborations in a model in vitro framework. Approach: Solutions containing 1.0 mg/ml type I collagen + 2.0 mg/ml HA (Elastin Products) in 0.1 M phosphate support, pH 7.4 were illuminated from 0 – 200 min. in a thermostatted cuvette (Hellma Cells) with a 4 W sifted UVG – 11 hand light discharging at 254 nm. Dityrosine arrangement as an element of time was checked by its fluorescence at excitation/discharge frequencies 325/400 nm for temperatures between 13 – 60 oC. Results and Discussion: For $T < T_m$ (~ 36 oC) HA impedes the pace of dityrosine development by ~ 20 – 30 %, demonstrating adjustment of collagen framework by HA. At $T > T_m$, where the wound adaptation commands, there has all the earmarks of being no methodical impact of HA on collagen solidness. In this manner, adjustment of collagen helical structure is by all accounts one significant capacity of the ECM.