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Effect of temperature on photochemical and thermal changes in calf skin collagen solutions at physiological pH

ammalian dermal collagen is in close contact with extracellular matrix (ECM) molecules, most prominently hyaluronan (HA) and associated proteoglycan (PG) molecules. The HA – PG system forms a complicated network that provides support for resting tissues and can facilitate wound healing and act as signaling molecules. We have previously used fluorescence of type I collagen tyrosine residues to study the ground- and excited state chemistry that results in structural and molecular alterations that could have significant (deleterious) effects. We are now turning our attention to the effect of surrounding ECM on collagen. Our strategy is to build up the ECM system starting with the core HA molecule, first adding glycosaminoglycans, (GAGs) then PG model compounds in solution. In this communication, we present the results of the effect of hyaluronan on collagen photo stability as evidenced by the rate of di-tyrosine formation as measured by di-tyrosine fluorescence ($\lambda ex =$ 325 nm, $\lambda em = 400$ nm). The experiments reported here consisted of model solutions of type I calfskin collagen (1 mg/ml) + hyaluronan (2 mg/ml) in 0.1 M phosphate buffer, pH 7.4 solutions were irradiated in a thermostatted quartz cuvette with a 4 W low pressure Hg lamp (main emission wavelength 254 nm superimposed on a low continuum). Temperature was varied from 8-62 \Box . The rates of dityrosine formation were monitored by the rise in 325/400 nm fluorescence with time. The effect of HA was determined by comparing the rates of fluorescence increase companion solutions of collagen + HA, expressed as the rate of R = collagen/collagen + HA as functions of temperature. Below the melting temperature Tm, R > unity and at 35°C and > 50°C, R < 1.0. Above 50°C, there is a rapid increase in di-tyrosine formation, and R~ unity. Our interpretation is that at lower temperatures, where the helical form exists, collagen is stabilized by HA; above the melting temperature, collagen is destabilized. At $T > \sim 50^{\circ}$ C, collagen exists as a denatured coil facilitation, with an activation energy that suggests hydrogen bond breakage. At body temperature R~ unity, suggesting that under physiological condition, there is minimal effect of HA on collagen.

Biography

Julian M Menter has received his PhD degree in Chemistry from the George Washington University in 1969. He has completed a Postdoctoral Fellowship with Prof. Dr. Theodor Foerster at the Institut fuer physikalische Chemie der Universitaet Stuttgart, Germany. Subsequently, he was at the University of Alabama, Birmingham, and the VA Medical Center (Atlanta). He currently serves as Research Professor of Biochemistry at Morehouse School of Medicine. He is recognized internationally for his work in areas of collagen photochemistry and melanin photobiology as pertaining to redox reactivity.

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