SUPPLEMENTAL DATA

Calculation of pentosidine formation rate constant (k_F) in collagen, aggrecan (A1D1) and its G1-domain (A1D6)

As described in ref [11], Appendix A, Equation 2, we use a mass balance equation in which the rate of accumulation of pentosidine with age is equated to the difference between the rates of pentosidine formation and protein turnover (i.e. pentosidine removal). The rate constant of pentosidine formation, k_F, can be calculated as:

k_F = [pentosidine/Lys](k_T) + d[pentosidine/Lys]/dt (Eq. 1)

where:

k_T is the rate constant of the protein turnover based on the racemization of aspartic acid (Table 1) [15, 16]; [pentosidine/Lys] is the concentration of pentosidine at a given age divided by the concentration of lysine (both are expressed as nmole/gr protein); and d[pentosidine/Lys]/dt is the increase in pentosidine per year divided by the concentration of lysine (Table 1). The small increase in protein content of aggrecan with age [15, 16] is neglected, as including this effect introduces a negligible change in the calculated rates of pentosidine formation [15, 16]. Since pentosidine formation is in the nanomole range, the amount of Lys residues available for crosslinking is not considered to change with age.

The increase of pentosidine per year, (d[pentosidine]/dt), was derived from the slopes of the best-linear fits to the graphs of pentosidine accumulation as a function of age. For collagen, an increase of 0.66 nmole (gr protein)$^{-1}$ year^{-1} was found for NP and AF (Fig. 1); for A1D1 no increase was observed (d[pentosidine]/dt=0), whereas for A1D6, an increase of 0.224 nmole (gr protein)$^{-1}$ year^{-1} was found (data not shown). Pentosidine concentration [pentosidine] in nmole/gr protein was taken from Fig. 1 for collagen and from Fig. 2 for A1D1 and A1D6.

Lysine concentration was taken as 1.04×10^{-4} mole/gr protein for A1D1 (28 residues per 268 kD protein), 2.19×10^{-4} mole/gr protein for A1D6, the aggrecan G1-domain (8 residues per 36 kD protein and 2×10^{-4} mole/gr protein for collagen (60 residues per 300 kD protein) [30]. Lysine concentration was found to be constant with age (data not shown). K_T values (Table 1) were taken from our previous data [15, 16].

In order to estimate the mean k_F value for collagen, A1D1 or A1D6, k_F was calculated for each age and finally averaged throughout the different ages sampled for each of the fractions. Mean k_F are presented in Table 1. For example, for A1D1 obtained from normal disc of 62 years, k_F was calculated using: $k_{T(A1D1)}$ of 0.134 $year^{-1}$ (Table 1); $[pentosidine]_{(A1D1)}$ of 1.67 nmole/gr protein (Fig. 2) and $[Lys]_{(A1D1)}$ of 1.04×10^{-4} mole/gr protein; d[pentosidine]/dt is zero. Thus:

$$k_{F(A1D1)} = 0.134 \times (1.67\times10^{-9} / 1.04\times10^{-4})$$
$$= 2.15\ \mu mol\ pentosidine\ (mol\ Lys)^{-1}\ year^{-1}$$

For A1D6 obtained from normal disc of 62 years, k_F was calculated using: $k_{T(A1D6)}$ of 0.033 $year^{-1}$ (Table 1); $[pentosidine]_{(A1D1)}$ of 14.1 nmole/gr protein (Fig. 2) and $[Lys]_{(A1D1)}$ of 2.19×10^{-4} mole/gr protein; d[pentosidine]/dt is 0.2247×10^{-9} nmole (gr protein)$^{-1}$ $year^{-1}$ Thus:

$$k_{F(A1D6)} = 0.033 \times (14.1\times10^{-9}/2.19\times10^{-4}) + (0.2247\times10^{-9} / 2.19\times10^{-4})$$
$$= 3.15\ \mu mol\ pentosidine\ (mol\ Lys)^{-1}\ year^{-1}.$$