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EVIDANCE FOR THE GLYCATION OF COLLAGEN BY SENSITIVE CAPILLARY ELECTROPHORESIS SEPARATION

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SUMMARY

Previous studies demonstrate that collagen in diabetes and aging undergoes extensive pos-translational modification by reducing carbohydrates resulting in decreased solubility and susceptibility to protease, increased stability and accelerated cross-linking, fluorescence and browning. Therefore, the elucidation of the nature of extracellular matrix cross-linking in aging and diabetes is of both clinical and theoretical interest.

In the present work the formation of cross-links in collagen, as a consequence of the *in vitro* glycation with reducing several reducing sugar has been studied, and the glycation compounds involved in these links have been separated and characterized by capillary electrophoresis technique.

Collagen type I was solubilizated in acetic acid 0.5 M and extensively digested with 1mg/ml pepsin. The collagen soluble fraction was incubated with 200 mM ribose, 200 mM fructose and 200 mM glucose respectively for 6 weeks in PBS pH 7.4 at 37^{o}C. The formation of the advanced glycation end products-AGEs and the crosslinking of collagen were assessed by labeled of protein with fluorescein isothiocyanate (FITC) and capillary electrophoresis (ABI 310 Prism) of fuorescein FITC adducts.

After 6 weeks of glycation capillary electrophoresis pattern of the glycated collagen showed the appearance of new peaks, corresponding to higher molecular weight at the comparing with unglycated collagen and the simultaneous disappearance or decrease in the height of the lower molecular weight peaks. This effect was even more evident after collagen incubation with ribose and fructose. In the case of glucose the modification in glycated collage molecular weight was lower.

The results of the present study indicate that *in vitro* glycation of collagen induces aggregation and cross-linking at a rate dependent on the type of reducing sugar, changes which can be successfully characterized by the capillary electrophoresis technique described by us.