ABSENCE OF MATURATION OF COLLAGEN CROSSLINKS IN FISH SKIN?

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1. Introduction

The maturation of collagen crosslinks in intact tissues can be studied by measuring hydrothermal isometric tensions (HIT) while the temperature is raised linearly as a function of time [1]. In particular, the presence in such tissues of collagen crosslinked with reducible heat-labile bonds is easy to differentiate from that of heat-stable networks [2]. In the presence of heat-labile crosslinks, continuous relaxation follows the recording of an early peak. On the other hand, when enough heat-stable crosslinks are present, the curve culminates close to or at boiling point.

The same types of heat-labile and heat-stable reducible crosslinks have been identified in the tissues of all the animal kingdom [3–5] whereas the nature of the heat-stable unreducible bonds which form multichain crosslinks in mammalian tissues with time is still unknown [6]. The latter crosslinks explain the insolubility of collagen in mature tissues. They are even formed in rat skin collagen despite its high solubility [7], and we have earlier observed their formation throughout maturation and aging, as reflected by the development of the HIT curves [8].

These experiments show that collagen crosslinks do not mature to thermally-stable bonds in fish skin, since the high-temperature tension region of the HIT curves, fails to develop during in vivo aging.

2. Materials and methods

2.1. *Tissues*

The fish were killed and immediately scaled. Their skins were carefully freed from foreign tissues, and kept air-tight at –20°C until use. The different species tested are listed in table 1. The tetrapods, frogs, salamanders and rats were raised in this laboratory and the skin treated as above.

2.2. *Age determinations*

The age of the fish was either known, because they had been raised in our laboratory, or determined by studying their scales [9]. When necessary, age difference was determined by the length and weight of fish caught on the same day at the same place.

2.3. *HIT measurements*

HIT curves were obtained as in [1], by preventing the swelling of the skin samples while the temperature was raised from 25°C to boiling point at a rate of 1.15°C/min.

3. Results and discussion

The HIT technique has already been used to study the maturation of collagen in rat skin [8] and in human skin [10]. When compared to these results, the HIT curves obtained from fish skin present some similarities and some differences.

First of all, the temperature at which the tension starts to develop (table 1) varies with the species and donor-age under study. These differences agree with [11], since tensions are known to develop with the denaturation of the collagen triple-helix [12]. The denaturation temperature increases with the stability of the triple-helix, which is linked: (i) to the amount of pyrrolidine-ring amino acids present in the molecules which varies from species to species [13]; and (ii) to the diameter of the collagen fibrils and fibres [12] which increases during maturation.

The skin of fish <2 years old so far examined gave similar curves (fig.1) whatever the species (sole, carp,

Table 1
Temperature observed for the starting point and maxima of the HIT curves obtained from the skin of fish of different ages and species

Species	Usual longevity (years)	Maximum longevity observed in captivity (years)	Origin	Age (years)	HIT results: Starting temp. (°C)	HIT results: Early max. temp. (°C)	Late max.
Plaice (*Pleuronectes platessa*)	?	25 [16]	Roscoff	> 4	43	51.4	absent
Dog-fish (*Scylliorhinus canicula*)			Roscoff	> 4	45.0	50.9	absent
Dog-fish (*Scylliorhinus canicula*)	7	8 [16]	Roscoff	> 7	45.8	52.8	absent
Sole (*Solea* sp.)	4/8	? [17]	Roscoff	≃ 1	46.3	52.8	present
Pike (*Esosc lucius*)	7	10/14 [16]	Seine river	> 1–2 <	48.6	56.5	present
Cat-fish (*Ictalurus nebulosus*)	8	? [18]	Seine river	> 2–3 <	50.2	59.3	present
Carp (*Cyprinus carpio*)	9/15	47 [16]	Laboratory	1.1	50.0	57	present
Carp (*Cyprinus carpio*)			Laboratory	6.1	51.8	60.2	absent
Hemichromis (*Hemichromis bimaculatus*)	?	5/6 ? (our obs.)	Laboratory	1.2	52	65.3	present
Frog (*Rana esculenta*)	4/5	6 [16]	Laboratory	3.0	44.7	absent	present
Salamander (*Salamandra salamandra*)	9	9/24 [16]	Laboratory	4.5	43.5	absent	present
Rat	2.5	3	Laboratory	2.5	59	absent	present
Human	40/70	110	Surgery	3.0	61	absent	present

catfish, pike). These curves displayed 2 maxima: an early peak (51–65°C, table 1), generally with a shoulder, and a late maximum close to the boiling point. For older fish, >2 years (carp, plaice, dog-fish), the HIT curves displayed a single, early peak with tension dropping almost to zero long before the boiling point was reached (fig.2). This development has much in common with that observed for rat skin between birth

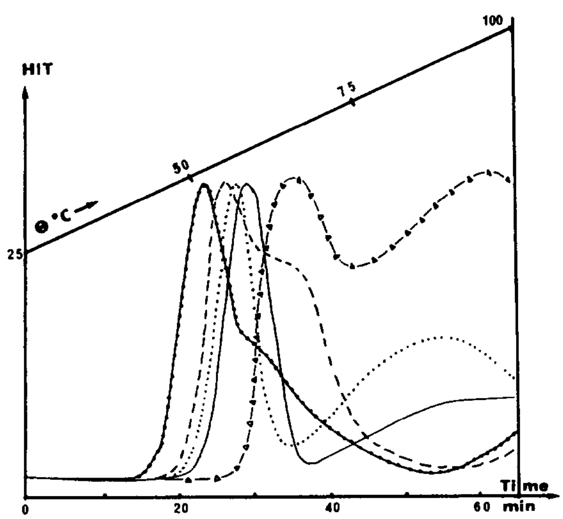

Fig.1. Examples of HIT curves for the skin of fish under 3 years. Compared to the HIT curve for 3 weeks old rat skin: (–•–) sole ≃ 1 year; (–△–) rat 3 weeks; (– –) pike > 1–2 years <; (. . .) carp 1.1 year; (——) cat-fish > 2–3 years <.

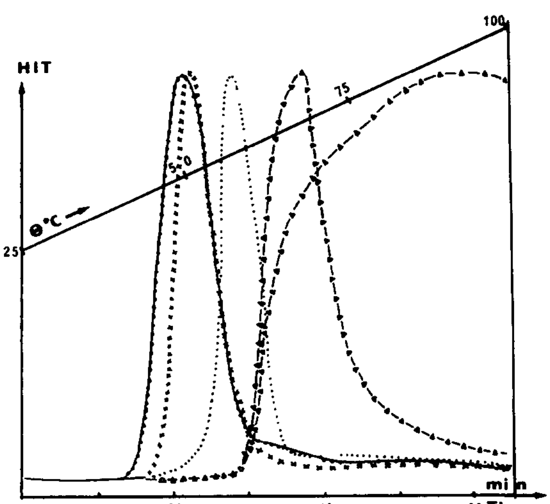

Fig.2. Examples of HIT curves for the skin of fish over 3 years. Compared to the HIT curves for the skin of 3 months and 2.5 years old rats: (–•–) plaice > 4 years; (–△–) 3 month old rat; (x x) dog-fish > 7 years; (–▲–) 2.5 year old rat; (. . .) carp 6.1 years.

and 9 months of age for which 2 maxima are present on the HIT curves in early life, while later on the first maximum alone is observed [8]. The early maximum was shown to be due to the presence and destruction of heat-labile reducible crosslinks in the collagen from rat skin [2], while the late maximum was linked to the presence of heat-stable reducible crosslinks in this tissue [2,8]. These latter bonds are known to disappear from the skin sometimes after birth, depending on the species [4] leading to the disappearance of the late maximum from the HIT curves. Similar modifications in the reticulation of collagen very probably occur in fish skin, since heat-labile reducible crosslinks have already been identified in this tissue [3,4], while the presence of heat-stable reducible bonds was only sometimes reported; however the age of the fish were not reported [3].

Contrary to expectation, the shape of the HIT curves obtained from fish skin do not seem to undergo any further major modifications during aging (fig.2). This is very surprising, since the last part of the HIT curves was shown to develop with years for man [10] as well as for rat skin [1,2] so that by 2–3 years of age the early peak has disappeared from the curves (fig.2). Similar curves have been obtained from the skin of all the tetrapods >3 years old we have studied (frog, salamander, rat, guinea-pig, monkey and man). All these animals display a profile similar to a 2.5 year old rat as illustrated in fig.2 which is known to indicate the presence in collagen of a heat-stable network. This heat-stability is the result of the transformation of the reducible heat-labile crosslinks into unreducible heat-stable bonds [14]. Such crosslinks apparently do not form in fish skin with time (table 1, fig.2) and the heat-labile crosslinks remain unchanged, thus causing the well known persistent high solubility of fish skin collagens [15].

Since the maturation of collagen is similar in rat and human skin (in preparation), the life-time of the different species (table 1) is certainly not the main factor involved in the pecularity of collagen reticulation in older fish. This factor could be the rate of aging, but in that case it would be extremely slow for collagen in fish skin.

Acknowledgements

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