Amino Acids (1993) 4: 1–3

Amino
Acids

Racemization rates of amino acids for dating ancient samples

Editorial

G. Lubec and **B. Lubec**

Clinical Biochemistry, The Hospitals for Sick Children, London, U.K.

Accepted July 24, 1992

Goodfriend proposes the use of rapid racemization of aspartic acid for dating (Goodfriend, 1992). We think that as the application of any amino acid dating system also this system has its values and limitations. We applied amino acid racemization on a series of ancient hair samples and would like to comment on the many factors influencing amino acid racemization based upon these observations and experimental data, making this type of dating unreliable for individual samples, though useful for a group of samples. We have, however, data in support of Goodfriend's work. And a confirmed paper is worth thousand unsupported.

We studied structural stability of hair from the eldest samples, a predynastic man, 3200 years BC to Egyptian mummies 18th–25th dynasty to copts (Egyptian christians) from 8–10th century graves (Lubec et al., 1987). Structural stabilities of hair samples were unchanged in terms of x ray diffraction and infrared studies. Racemization that must have been taken place over this long period was evaluated using amino acids involved in the determination of the conformation of structural proteins: proline and hydroxyproline; their racemization i.e. D/L ratios and isomerization i.e. cis-trans hydroxyprolines were examined. Recent hair samples served as controls. The analyses were carried out by high pressure liquid chromatography (Kampel et al., 1990) and quantitative fluorescence thinlayer chromatography controlled by GC/MS (Lubec et al., 1992).

The results are given in the figure showing that the oldest sample's racemization rate (D/L proline) was found in the range of mummies. Needless to say that an overlap of this dimension would limit the use of amino acid dating. Two out of the ten copts examined showed values close to the mummies' data. The isomerization of trans to cis 4 hydroxyproline, however, clearly separated the three different periods. Can we assume that racemization and isomerization have two different chemical underlying mechanisms?

The predynastic man buried in the hot dry sand that preserved his body for approx 5200 years and racemization values resembling those of mummies pre-

served in a clearly different atmosphere put the values of temperature studies of Goodfriend, heating Negev snails at 106.5 °C, as well as temperature as a main factor in question. Why do two out of 10 copts of the same cemetery and presumably the same temperature present higher racemization values (but not isomerization values)? Temperature is not the explanation (also not for the racemization of aspartic acid by heating snails for days, a catalyst must have been present, otherwise only under strong acidic conditions as hydrolysis high racemization rates could be expected). Environmental factors are not likely to be responsible. Known influences as pH, ionic strength, chemical environmental influences (copts were found in quartz, acid pH) should have been comparable in the coptic panel. Microbial influences on racemization seem to be the most probable explication. Racemases and D amino oxidases are abundant and the origin is clear: the human intestinal normal flora is the major source of enzymes acting on racemic amino acids thus influencing D/L amino acid ratios. Marine algae are another major ecological supplier of those enzymes (Adams, 1972). It is obvious that washing hair or shells or snails in any solution would not be able to abolish enzyme activities having worked for centuries. Dr. Goodfriend stated

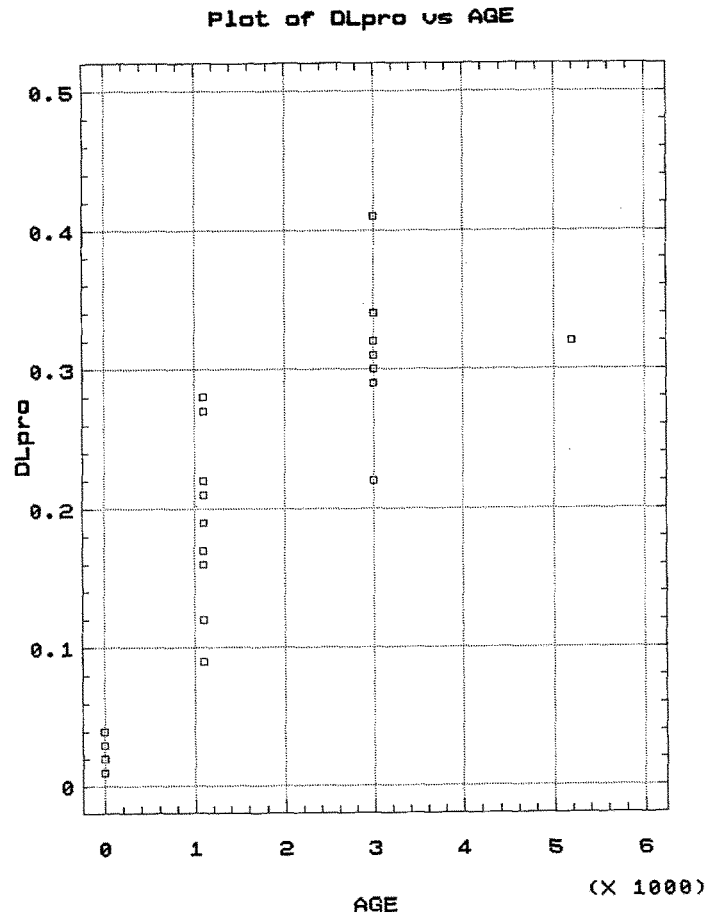

Fig. 1. The D/L ratio of proline is plotted versus the age of recent men ($n = 10$), copts ($n = 10$), Egyptian mummies ($n = 11$) and a predynastic man

that variation in racemization values among species could be another factor. It is. At least in the mammalian species. Hair of dogs differs in racemization and isomerization of proline from rats and mice and man, dogs showing the lowest and man the highest racemization rates and even hair from different areas of the human body presents different D/L hydroxyproline ratios (author's observation). One can of course claim that hair is not mollusc shells but what has been examined in both cases is structural proteins hydrolyzed to amino acids. I do not wish to comment on the many factors influencing D/L amino acid ratios by hydrolysis procedures: differences in the vials (glass ware) used can be crucial but it serves as a model for factors influencing racemization: the presence or absence of metal ions in or on the samples to be dated, known to catalyse racemization must be taken into account. Dating of samples exposed to sunlight/daylight cannot be compared to dating of samples in graves as major factors for racemization are all kinds of radiation, ionizing and nonionizing, from uv to microwaves.

The former might be of minor relevance to samples examined by Dr. Goodfriend as the structural proteins in the shell would have been physically and chemically well protected by the calcium carbonate embedding. Nevertheless, does not the kinetics of racemization point to a role for radiation over a period of long?

A main influencing factor is the different susceptibility of individual proteins towards racemization of their building blocks, the amino acids as well as enormous naturally occurring differences: soy bean protein—as known to the food chemist—has the highest degree of D amino acid composition of all animal or plant proteins.

Our own data showing excellent correlation between D/L racemization and age from recent men to Egyptian mummies (provided groups are statistically evaluated), are supporting Dr. Goodfriend's findings but the many influences limit the use for dating individual samples, thus warning archaeologists who do not find groups of predynastic men that can be statistically handled.

References

Adams E (1972) Racemases and epimerases. In: Boyer E (ed) The enzymes, vol. 6. Academic Press, New York, pp 479–507

Goodfriend GA (1992) Rapid racemization of aspartic acid in molluscs. Nature 357: 399–401

Kampel D, Kupferschmidt R, Lubec G (1990) Toxicity of D-proline. In: Lubec G, Rosenthal G, (eds) Amino Acids, Escom, Leiden, pp 1164–1171

Lubec G, Nauer G, Seifert K, Strouhal E, Porteder H, Szilvassy J, Teschler M (1987) Structural stability of hair 4000 years old. J Arch Science 14: 113–120

Lubec B, Vycudilik W, Mallinger R, Hausler J, Popow C, Lubec G (1992) Antigenic changes of the glomerular basement membrane after the incorporation of cis-hydroxyproline isomers. Renal Physiology 15: 33–40

Authors' address: Prof. Dr. G. Lubec, Chem, FRSC, The Hospitals for Sick Children, Clin. Biochemistry, Great Ormond Street, London WC1N 3JH, U.K.

Received May 1, 1992