

The Inverse Relationship between Tryptophan and Hydroxyproline in Animal Tissues

OLLE DAHL

Scan's Centrallaboratorium, Malmö, Sweden

During recent years several contributions have increased our knowledge of the amino acid composition of proteins and in particular their content of essential amino acids.

Tryptophan is an essential amino acid typical of high-quality animal proteins and may often, although not always, be considered a special feature of this kind of

protein. Blood protein is an example of a protein high in tryptophan but low in the essential amino acid methionine¹. This is in contrast to, for instance, striated muscular tissue, milk, and egg protein.

An amino acid typical of the low-quality protein present in connective tissue is hydroxyproline, the assessment of which has acquired increased importance over the past few years. Since connective tissue is practically devoid of tryptophan and, on the other hand, striated muscular tissue contains very little hydroxyproline, there is an indication of an inverse relationship between tryptophan and hydroxyproline. The present study was undertaken to assess, whether this relationship is a general characteristic of animal tissues or is subject to limitations. In particular, unstriated muscular tissue, *i.e.* the tissue found in stomach, intestine etc., was investigated.

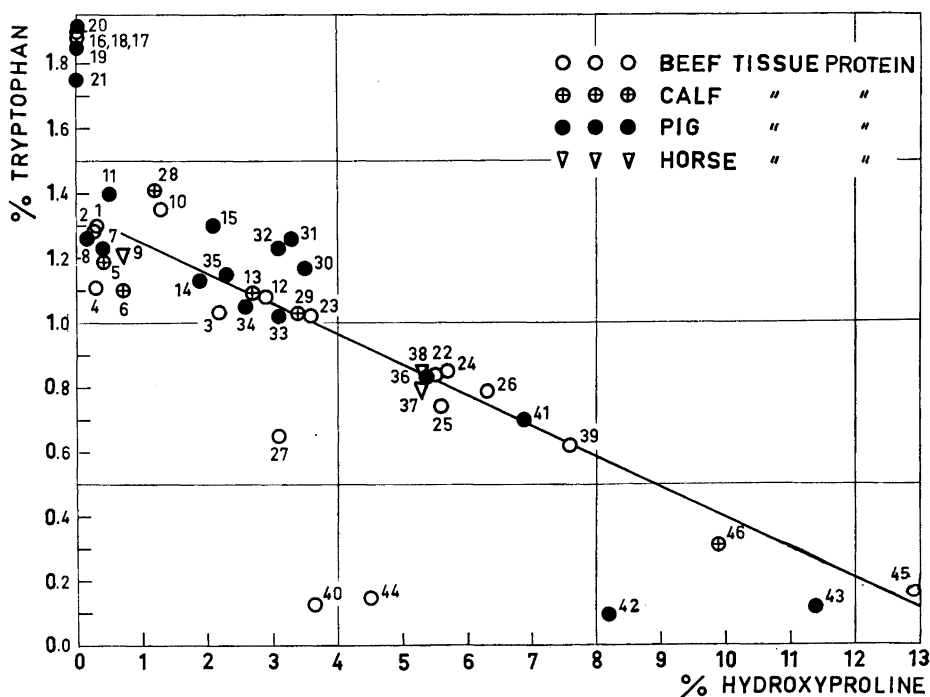


Fig. 1. Relation between the content of tryptophan (T) and hydroxyproline (H) in various animal tissues. The proportions of the two amino acids are given as percentages of total protein ($N \times 6.25$).

The regression line drawn, $T = -0.0950 \cdot H + 1.345$ has been calculated on the basis of all the data except those indicated in the text; coefficient of correlation -0.949 , significant at more than the 99.9 % level.

Key to Fig. 1:

1. Longiss. dorsi (1); heifer, 26 months, grade expr.
2. Longiss. dorsi (1); cow, 8 years, grade 3+.
3. Beef meat, assortment III (fairly rich in connective tissue); cow, 7 years, grade 2+.
4. Longiss. dorsi (1); bull, 11 years, grade expr.
5. Longiss. dorsi (2); calf (fattened, male), 9 weeks, grade 1+.
6. Longiss. dorsi (2); calf (newly born, male), < 1 week.
7. Longiss. dorsi (3); bacon pig (hog), 6½ months, grade expr.
8. Longiss. dorsi (3); sow, 2 years, grade 1.
9. Horse meat, assortment II (fairly poor in connective tissue); animal 11 years old, grade 1.
10. Spleen, cow
11. » pig
12. Lungs, cow
13. » calf (fattened)
14. » pig sample No. 1
15. » pig sample No. 2
16. Blood, beef
17. Blood plasma, beef
18. Blood corpuscles (4), beef
19. Blood, pig
20. Blood plasma, pig
21. Blood corpuscles (4), pig
22. Rumen, cow
23. Omasum, cow
24. Fat end (Rectum anal end), cow
25. Udder, cow
26. Mouths (without skin), cow
27. Glands from cow's head
28. Small intestine (round), calves (fattened)
29. Fat ends (Rectum anal ends), calves (fattened)
30. Stomach (maw), pig, sample No. 1
31. » (»), » » » 2
32. » (»), sow
33. Caps (Caecum), pig
34. Chitterlings (large intestine, colon), pig, sample No. 1
35. Chitterlings (large intestine, colon), pig, sample No. 2
36. Bladders, pig
37. Stomach, horse
38. Bung (Caecum), horse
39. Cracklings (connective tissue) from wet rendered beef tallow (5)
40. Cracklings (connective tissue), residue from solvent extracted perinephric beef tallow
41. Cracklings (connective tissue), from wet rendered pig fat (5)
42. Cracklings (connective tissue), residue from solvent extracted pig's back fat
43. Back rind, pig
44. Neck tendon (ligamentum nuchae), cow
45. Achilles tendon, cow
46. Achilles tendon, calves (fattened, male)

(1) From the last rib to the second lumbar vertebra.

(2) From the last dorsal vertebra to the second lumbar vertebra.

(3) Between the last three (12th to 14th) dorsal vertebra.

(4) Residue after separation of blood into plasma and corpuscles [proportion of plasma to corpuscles 7:3 (beef blood) and 6:3 (pig blood), respectively].

(5) Commercial fatty raw material containing intestinal glands.

This kind of tissue, which is of a considerable interest from a nutritional and also physiological point of view, has not been previously investigated for its tryptophan and hydroxyproline contents.

Tryptophan was determined on the minced tissue without previous hydrolysis according to the widely used method of Bates essentially as described by Graham *et al.*³ Hydroxyproline was assessed according to the method of Neuman and Logan as modified by Stegemann². This was compared with the modification of Lollar⁴ for different kind of tissues. The agreement between the methods was good. The results appear in Fig. 1.

With a few exceptions a close linear, negative correlation was found to exist between the contents of tryptophan and hydroxyproline. The regression line was

calculated on the basis of all the data except those of the blood and blood fractions (samples Nos. 16–21), which are very high in tryptophan but contain no hydroxyproline, and the samples No. 27 (glands), Nos. 40 and 42 (cracklings from fatty tissue), and No. 44 (*ligamentum nuchae*).

The inverse relationship between tryptophan and hydroxyproline could best be explained by assuming tryptophan to be related to true cellular, and hydroxyproline to connective tissue content. This point is being investigated, as is the unforeseen difference in hydroxyproline between young and old animals (*cf.* samples Nos. 45 and 46).

The contents of tryptophan and hydroxyproline in unstriated tissues (Nos. 28–38) are intermediate. This is, among other

things, of interest from a nutritional point of view, since stomachs, intestines, and udders are used by some food manufacturers either alone or mixed with other ingredients in various chopped meat products.

The assistance of Mrs. Siv Olsson, and Mrs. Anni Esping is gratefully acknowledged.

1. Block, R. J. and Weiss, K. W. *Amino Acid Handbook*, Charles C. Thomas, Publisher, Springfield, Illinois, U.S.A.; 1956.
2. Graham, C. E., Smith, E. P., Hier, S. W. and Klein, D. J. *Biol. Chem.* **168** (1947) 711.
3. Stegemann, H. Z. *Physiol. Chem. Hoppe-Seyler* **311** (1958) 41.
4. Lollar, R. M. J. *Am. Leather Chemists' Ass.* **53** (1958) 2.

Received January 18, 1960.

The Solubility and the Salt Sensitivity of Yeast D- and L-Lactic Cytochrome c Reductase

AGNAR P. NYGAARD

Johan Throne Holst's Institutt for Ernæringsforskning, Blindern, Oslo, Norway

Homogenates of aerobic yeast have been found to contain D- and L-lactic cytochrome c reductase in the ratio 1:2^{1,2}. Using our extraction method / (15–30°), mainly the L-enzyme was solubilized. At low temperatures (0–5°) both enzymes were obtained in the particulate fraction. Half or more of the L-activity was readily extracted from the particles at 30° with 0.2 M disodium phosphate, whereas the D-enzyme remained insoluble. The extracted particles contained D- and L-lactic cytochrome c reductase in the ratio 1:1. Further mechanical desintegration with ballotini beads gave rise to soluble D-lactic cytochrome c reductase; very little of the L-enzyme was obtained. Complete extraction was not achieved for any of the two enzymes. It is concluded that one part of the L-activity was more firmly bound to the particulate fraction than the other.

Following the described method³, three fractions of lactic cytochrome c reductase were obtained^{2,4}. Fractions I, II, and III were eluted in that order from a N,N-diethylaminoethyl-cellulose column. Fractions I and II were specific for L-lactic acid. Fraction III was D-specific (D-LDH III).

However, small amounts of a L-specific enzyme has now been found in some of the fractions III (L-LDH III).

The D- and the L-enzyme of the cell homogenate were both salt sensitive. The D-enzyme was more salt sensitive than the isolated D-LDH III². The L-enzyme was inhibited 50 % when the phosphate concentration (pH 7.1) was increased from 0.01 to 0.08 μ .

The activity of fractions I and II, which represented most of the L-LDH activity of the purified solution, was quite the same in phosphate of ionic strength 0.01 and 0.08. In contrast, both D- and L-LDH III were inhibited 70–90 % when the buffer concentration was increased from μ 0.01 to 0.08. The inhibition of L-LDH III was the same with low and high concentrations of cytochrome c, whereas the inhibition of D-LDH III was less at high concentrations of the acceptor².

The strong effect of salt on L-LDH of the cell homogenate suggests that fair amounts of the salt sensitive enzyme is present in the cell. In this connection it is interesting that L-LDH obtained by the method of Boeri *et al.*⁶ is very salt sensitive.

To the authors' knowledge, the effect of salts on D- and L-LDH III is stronger than for any other enzymes described. From other relationships D-lactic cytochrome c reductase has been proposed as intermediate in the formation of the L-enzyme^{1,5}.

D- and L-LDH III were eluted together with ribonucleic acids⁷. Furthermore, the enzymes precipitated together with nucleic acids when the eluate was dialyzed against water. A charge aggregate between protein and nucleic acid might explain both the salt sensitivity and the high negative charge which these two enzymes have available to the anion exchanger.

1. Nygaard, A. P. *Arch. Biochem. Biophys.* *In press.*
2. Nygaard, A. P. *Biochim. et Biophys. Acta.* *In press.*
3. Nygaard, A. P. *Biochim. et Biophys. Acta* **33** (1959) 517.
4. Nygaard, A. P. *Biochim. et Biophys. Acta* **35** (1959) 212.
5. Nygaard, A. P. *Arch. Biochem. Biophys.* *In press.*
6. Boeri, E. and Tosi, L. *Arch. Biochem. Biophys.* **60** (1956) 463.
7. Nygaard, A. P. *Biochim. et Biophys. Acta* **30** (1958) 450.

Received January 2, 1960.