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Phil. Trans. R. Soc. Lond. B 1970 **257**, 147-151
doi: 10.1098/rstb.1970.0017

Phil. Trans. Roy. Soc. Lond. B **257**, 147–151 (1970) [147]
Printed in Great Britain

The N-terminal and C-terminal amino acid sequence of calf rennin

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The enzyme rennin (EC 3.4.4.3) is well known as the milk clotting enzyme from the fourth stomach of the calf. It appears, however, that the enzyme may be regarded as a suitable model enzyme for studies of the structure and function of gastric proteases.

The general properties of the enzyme have recently been reviewed (Foltmann 1966), and the amino acid sequences of some soluble tryptic peptides and the disulphide bridges have been investigated (Foltmann & Hartley 1967). In the latter paper the only soluble tryptic peptides that were investigated were basic or neutral. Subsequent experiments have shown that two acidic peptides, which only appeared with a modest ninhydrin reaction in the first experiments are in fact significant fractions of the tryptic digest. One of these peptides represents the N-terminal amino acid sequence, while the other is a part of the C-terminal amino acid sequence.

The N-terminal sequence

Jirgensons, Ikenaka & Gorguraki (1958) have earlier reported that glycine is the N-terminal amino acid residue of rennin. This was confirmed by analysis of a chymotryptic digest of rennin which had been reacted with maleic anhydride (Butler, Harris, Hartley & Leberman 1967). In this reaction partial amides are formed between maleic acid and free amino groups in the protein, i.e. N-terminal amino groups and ϵ-amino groups of lysine. The maleyl groups may be removed again by treatment at pH 3.5, and as suggested by Butler *et al.* (1967) the peptides containing free amino groups may be isolated by diagonal electrophoretic techniques analogous to those described by Brown & Hartley (1966) and by Tang & Hartley (1967).

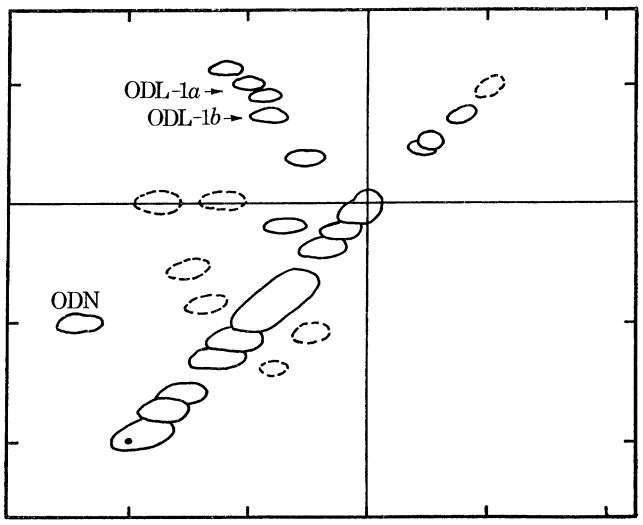

Figure 1. pH 6.5 diagonal electrophoretic map of a demaleylated chymotryptic digest of rennin. Conditions were as described in the text. Cathode to the right in the first dimension, and at the top in the second. • indicates a marker of l-dimethylaminonaphtalene-5-sulphonic acid.

The maleylated rennin was hydrolysed with chymotrypsin, and the digest was fractionated by high voltage paper electrophoresis at pH 6.5. A guide strip was cut from the sheet, and the amino groups were demaleylated by exposure to the vapours of a pyridine–acetate buffer (pH 3.5) at 60 °C for 6 h. The demaleylated strip was stitched to a second sheet of filter paper, and electrophoresis was performed at right angles to the first fractionation. The resulting diagonal map is shown in figure 1. Of the peptides lying off the diagonal, only peptides ODN, ODL-1 *a*, and ODL-1 *b* are considered in this paper. The appropriate zones from the preparative main sheet were cut out and demaleylated, and the peptides were isolated after electrophoresis at pH 6.5. The amino acid compositions of all peptides mentioned in this paper are compiled in table 1.

TABLE 1. COMPOSITIONS OF PEPTIDES FROM RENNIN

Compositions are expressed as uncorrected ratios of amino acids after 16 to 20 h hydrolysis in constant boiling HCl at 110 °C. The electrophoretic mobilities at pH 6.5 are expressed relative to that of 1-dimethylaminonaphthalene-5-sulphonic acid (−1.0), at pH 2 relative to DNS-arginine (1.0). The colour is after staining with cadmium-ninhydrin according to Heilmann *et al.* (1957).

	mobility at pH 6.5			
	1st dim.	2nd dim.	colour	composition
off-diagonal peptides from a chymotryptic digest of maleylated rennin, and fragments of these				
ODN	−1.35	−0.55	yellow	Ser(0.9), Glu(1.1), Pro(0.9), Gly(1.0), Ala(1.0), Val(1.9), Leu(1.1)
ODN-P 1	−0.8	—	yellow	Glu(1.0), Gly(1.0), Ala(1.0), Val(1.0)
ODN-P 2	−0.1	—	red	Ser(1.0), Pro(1.0), Val(1.0), Leu(1.0)
ODL-1 *a*	−0.55	+0.55	yellow	Lys(1.1), Gly(1.0), Ile(1.0), Tyr(0.9)
ODL-1 *b*	−0.5	+0.4	red	Lys(1.0), Gly(1.1), Ile(1.0), Tyr(0.8), Phe(1.0)
arginine peptide from a chymotryptic digest of maleylated rennin				
CA-4	0	(pH 2) 0.85	red	Arg(1.0), Glu(1.1), Ile(0.9), Tyr(0.9)
tryptic peptides of denatured rennin, and fragments of these				
H	−0.25	—	yellow	Lys(1.1), Asp(2.1), Thr(1.0), Ser(1.8), Glu(2.0), Pro(1.0), Gly(2.0) Ala(1.0), Val(2.0), Leu(1.9), Tyr(1.9), Phe(0.9)
HC-1	−0.55	—	yellow	Ser(0.9), Glu(1.1), Pro(0.9), Gly(1.0), Val(2.0), Leu(1.0), Ala(1.0)
HC-2	−0.1	—	orange	Asp(1.1), Thr(1.0), Tyr(0.8)
HC-3	−0.65	—	red	Asp(1.0), Ser(0.9), Glu(1.0), Leu(1.0), Tyr(0.7)
HC-4	+0.55	—	red	Lys(1.0), Gly(1.0), Phe(1.0)
HC-5	−0.4	—	red	Asp(1.1), Ser(0.9), Glu(1.0), Leu(0.9), Tyr(0.8), Phe(1.0)
HC-6	+0.75	—	yellow	Lys(1.0), Gly(1.0)
HC-7	−0.4	—	orange	Asp(2.0), Thr(0.9), Ser(1.0), Glu(1.2), Leu(1.0), Tyr(1.5)
J	−0.4	—	red	Arg(0.9), Asp(1.0), Ser(0.9), Glu(1.0), Val(1.0), Tyr(1.7), Phe(1.0)
JC-1	−0.7	—	red	Glu(1.0), Tyr(1.9)
JC-2	−0.1	—	orange	Ser(0.9), Val(1.0), Phe(1.0)
JC-3	0	(pH 2) 1.0	red	Asp(1.0), Arg(1.0)

Peptide ODN contains no lysine, and did not react with ninhydrin before unblocking. This shows that its α-amino group has been maleylated. Consequently we must conclude that peptide ODN represents the N-terminal amino acid sequence of the original protein. The peptide was sequenced by the 'dansyl-Edman' technique (Gray 1967) as shown in figure 2. After four Edman degradations the results became ambiguous, and an aliquot of the peptide was hydrolysed with papain. After purification by paper electrophoresis each of the two fragments obtained were sequenced as shown.

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Peptides ODL-1*a* and ODL-1*b* were hydrolysed with trypsin, and analyses of the fragments show that both peptides in fact represent the same lysine residue. Figure 2 shows the sequences of these peptides.

From a tryptic digest of rennin which had not been maleylated an acidic peptide (H) was isolated after paper electrophoresis at pH 6.5 and further purification by electrophoresis at pH 3.5. The peptide stained yellow with cadmium-ninhydrin, and consistent with this glycine was found as N-terminal residue by the 'dansylation' method. Peptide H was hydrolysed with chymotrypsin, and the individual fragments were purified by paper electrophoresis and sequenced as shown in figure 2.

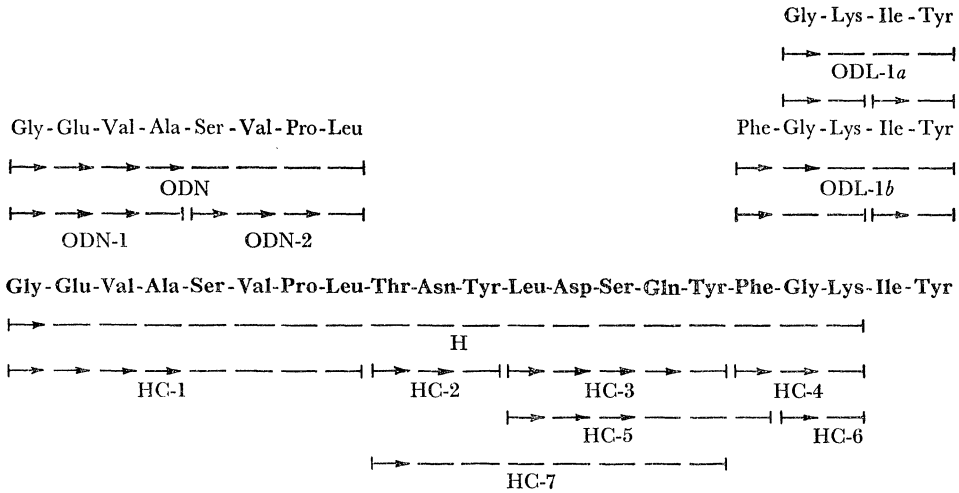

FIGURE 2. The N-terminal amino acid sequence of rennin. Peptides are numbered as in table 1, and figure 1. The symbol ——— indicates the quantitative amino acid composition of the peptide; ——→ indicates location by the 'dansyl-Edman' techniques (Gray 1967).

Since peptide H derived from a tryptic digest the sequence Gly-Lys could immediately be placed as the C-terminus. N-terminal glycine in the original peptide identified HC-1 as the N-terminal fragment, and the relative position of HC-2 and HC-3 was established by a separate limited chymotryptic digestion of peptide H from which peptide HC-7 was isolated.

Peptide HC-2 was almost neutral; hence asparagine was present. From its electrophoretic mobility peptide HC-3 could be expected to contain one amide group (Offord 1966) and after two cycles of Edman degradation the peptide was neutral after paper electrophoresis at pH 6.5; thus glutamine was present here.

It is obvious that peptide HC-1 is identical with peptide ODN, and this proves that peptide H in fact represent the N-terminal amino acid sequence of rennin. Further, the off-diagonal lysine peptides ODL-1*a* and ODL-1*b* overlap the C-terminus of peptide H, and this allows us to extend the N-terminal sequence to the 21 residues shown in figure 2.

THE C-TERMINAL SEQUENCE

In a previous communication (Foltmann & Hartley 1967) a C-terminal sequence of 13 residues was reported. From the homology with pepsin, it was predicted that one of the small insoluble tryptic peptides would be located close to the C-terminus. The missing fragment, and the connecting, overlapping sequence have now been isolated.

Peptide J which in paper electrophoresis at pH 6.5 moves just ahead of the N–terminal peptide H, was isolated from a tryptic digest of rennin and digested with chymotrypsin. The fragments were again purified by paper electrophoresis and sequenced as illustrated in figure 3. The order of the fragments is established by finding N-terminal Glu in the original peptide and in peptide JC-1, while JC-3 can be placed as C-terminus due to C-terminal arginine. The electrophoretic mobilities show that no amides are present in peptide J.

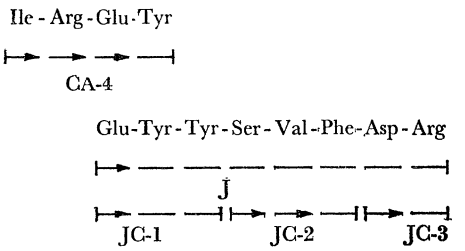

FIGURE 3. A part of the C-terminal amino acid sequence of rennin. Symbols are given as in figure 2.

An overlap connecting the N-terminal part of J to the preceding tryptic peptide is provided by a neutral arginine peptide CA-4 which was isolated from the chymotryptic digest of maleylated rennin (figure 3). The C-terminal Asp-Arg of peptide J overlaps the known C-terminal sequence (figure 4).

rennin	**Try-Ile - Leu - Gly - Asp - Val - Phe - Ile - Arg** - Glu - **Tyr - Tyr** - Ser - **Val**
pepsin	**Try-Ile - Leu - Gly - Asp - Val - Phe - Ile - Arg** - Gln - **Tyr - Tyr** - Thr - **Val**
rennin	**Phe-Asp - Arg - Ala - Asn - Asn** - Leu - **Val - Gly - Leu - Ala** - Lys - Ala - Ile -OH
pepsin	**Phe-Asp - Arg - Ala - Asn - Asn** - Lys - **Val - Gly - Leu - Ala** - Pro - Val - Ala-OH

FIGURE 4. The C-terminal amino acid sequence of rennin compared to that of hog pepsin (Dopheide, Moore & Stein 1967; Stepanov 1968).

HOMOLOGIES BETWEEN RENNIN AND PEPSIN

Altogether we now know 28 residues of the C-terminal amino acid sequence of rennin as shown in figure 4. This figure also includes the C-terminal amino acid sequence of hog pepsin (Dopheide, Moore & Stein 1967; Stepanov 1968). The two gastric proteases show a very pronounced homology in their C-terminal sequences, although it is remarkable that the only lysine residue present in pepsin is absent in rennin. The homology of the amino acid sequences around the S–S bridges reported previously (figure 5) makes it likely that large parts of the structures of gastric proteases are similar, but so far none of the published amino acid sequences from pepsin show any similarity to the N-terminal amino acid sequence of rennin.

Finally it should be added the crystalline rennin in fact is a mixture of at least three different components, which may be separated by chromatography on columns of DEAE-cellulose (Foltmann 1966). Most of the sequence work has been carried out with total crystalline rennin. However, peptide maps of the major component, chromatographically purified B-rennin, showed no obvious differences from those of total crystalline rennin. To what extent the N-terminal and C-terminal amino acid sequences of A- and C-rennin are identical with those of B-rennin remain to be investigated.

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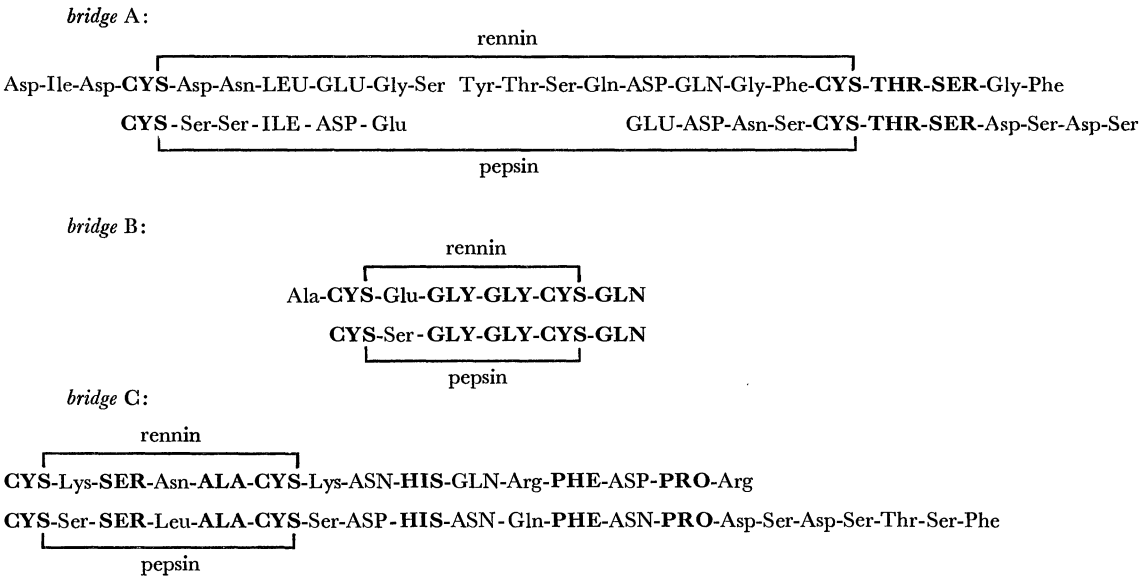

FIGURE 5. Comparison between the S–S bridges in rennin and pepsin. The rennin sequences of Foltmann & Hartley (1967) are extended in bridge C by a chymotryptic peptide, His-Gln-Arg-Phe which overlaps the tryptic peptide, Phe-Asp-Pro-Arg. The pepsin sequences are a private communication from J. Tang and B. S. Hartley. Chemically similar residues are in capitals and identical residues are in bold capitals.

The support of the Carlsberg Foundation is gratefully acknowledged.

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