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APPENDIX

Mathematical Derivation of the Yields of the Products of Hydrolysis of the Hexapeptide Ser.Gly.Ala.Gly.Ala.Gly

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The problem of relating the composition of a partial hydrolysate of a protein to its amino acid sequence and the hydrolysis constants of its constituent bonds is one of considerable interest to protein chemists, and has been discussed by Sanger (1952) and by Desnuelle (1953). Little progress has been possible in elucidating the problem because of the complex structure of most proteins, and because the rate of hydrolysis of any bond is likely to vary during the course of hydrolysis according to the particular peptide in which the bond occurs.

In the paper to which this is the Appendix, we have reported on a polypeptide (CTP) derived from silk fibroin; CTP is believed to be composed largely of the repeating hexapeptide units Ser.Gly.Ala.Gly.Ala.Gly. Consequently, although CTP has a molecular weight of approximately 4000, its structure is relatively simple, and the variety of its bonds is much less than normally occurs in proteins. Because of the lability of the Gly.Ser bond, this polypeptide would be expected to break down rapidly in concentrated acid to the simple hexapeptide units, and the yields of amino acids and peptides, which we have determined at various stages of the hydrolysis of the polypeptide, would

therefore approximate to those that would be derived from the hexapeptide unit.

As a contribution to the general problem therefore it seemed worth while to calculate the yields of the hydrolysis products by making some simple assumptions about the hydrolysis constants of the bonds. In addition to this it was hoped that the calculations would throw some light on a feature of the experimental results that was not obviously explained by the structure of the hexapeptide, namely the constant occurrence in the partial hydrolysate of Ala.Gly and Gly.Ala in the ratio 2:1.

It is known that polypeptides hydrolyse more rapidly than would be expected from the experimentally determined rates of their bonds in dipeptide form. It is supposed that this comparative stability of dipeptides is due to the effect of the charged NH_3^+ group, which tends to repel the H^+ ions, and, if this is so, it is reasonable to assume that an *N*-terminal peptide bond will also be more stable than the same bond when it occurs elsewhere within the peptide chain.

The rates of hydrolysis of the dipeptides Ser.Gly, Ala.Gly, and Gly.Ala were determined experimentally, the same conditions of hydrolysis being

used as for CTP, and the velocity constants calculated. These were: Ser.Gly, 5.6×10^{-3} (hr.⁻¹); Ala.Gly, 1.37×10^{-2} (hr.⁻¹); Gly.Ala, 1.37×10^{-2} (hr.⁻¹). The absolute rates of hydrolysis of the internal peptide bonds in a peptide chain are not known, and for the internal Ala.Gly and Gly.Ala bonds a rate of hydrolysis that gave the closest general fit to the experimental results was deduced by the method shown at the end of the mathematical section.

The assumptions adopted were therefore (a) that the *N*-terminal Ser.Gly bond has a constant rate of fission and a velocity constant of 5.6×10^{-3} (hr.⁻¹); (b) that the bonds in Ala.Gly and Gly.Ala, when these occur as the *N*-terminal bond or in free dipeptides, have an equal and constant rate of fission with a velocity constant of 1.37×10^{-2} (hr.⁻¹); (c) that the bonds in Ala.Gly and Gly.Ala, when they do not occupy an *N*-terminal position, break down at an equal and constant rate, with a velocity constant of 5.0×10^{-2} (hr.⁻¹).

By using these assumptions, we have obtained differential equations that give the rate of increase in the amounts of the various products of hydrolysis, and by solving these equations we have obtained the yields after various periods of time. Table 1 shows a comparison between the calculated values and the experimental values from the partial hydrolysis of the CTP. As the intervals of time used in the calculation do not coincide with those used in the experimental determinations, the comparable experimental values have been derived from smoothed curves of the rate of production of the appropriate products of hydrolysis. The calculated values given in the table have been reduced by 15% to enable a fairer comparison to be made with the experimental values, in the determination of which losses of this order occur.

The agreement between the two sets of results, though far from perfect, is reasonably good with regard to the simplicity of the assumptions made. The feature of greatest interest is the fact that, for periods of hydrolysis greater than 10 hr., the calculated values indicate a ratio of Ala.Gly to Gly.Ala of approximately 2:1, thus confirming the constancy of this ratio found experimentally.

Derivation of the equations

For simplicity the number of moles of each peptide present at time *t* hr. after the start of hydrolysis will be denoted by

SGAGAG, SGAGA, GAGAG, SGAG, etc. (1)

where S is serine, G is glycine and A is alanine.

Let *x* be the rate of fission of the *N*-terminal bond between S and G; let *y* be the rate of fission of the *N*-terminal bond between G and A, or between A and G; and let *z* be the rate of fission of these latter

Table 1. *Partial hydrolysis products of CTP or SGAGAG*

Yields of amino acids and peptides found (F) after hydrolysis of CTP for various times, and calculated (C) from the hexapeptide Ser.Gly.Ala.Gly.Ala.Gly, are compared. The found and calculated values are expressed as numbers of residues of amino acid or peptide/100 amino acid residues of CTP or hexapeptide respectively. The calculated values have been decreased by 15% (see text).

Time of hydrolysis (hr.)	Gly		Ala		Ser		Ala.Gly		Gly.Ala		Ser.Gly		Gly.Ala.Gly		Ser.Gly.Ala		Ala.Gly.Ala		Ala.Gly.Ala.Gly	
	F	C	F	C	F	C	F	C	F	C	F	C	F	C	F	C	F	C	F	C
5	1.8	3.3	1.4	0.8	0.7	0.4	4.6	3.1	2.1	0.6	3.4	3.0	2.2	2.0	1.7	2.1	0.8	0.5		
10	3.7	6.3	2.9	2.7	1.2	0.8	7.6	5.2	3.6	1.6	5.0	5.3	3.0	2.3	2.4	3.2	1.0	1.0		
20	7.5	11.0	5.6	6.7	1.9	1.5	10.6	7.4	5.2	3.2	7.0	8.0	2.4	1.7	2.2	2.9	0.9	1.1		
30	11.3	14.4	8.4	10.4	2.3	2.2	11.6	7.9	5.8	3.8	7.3	9.3	1.8	1.1	1.8	2.1	0.6	0.8		
40	15.0	17.5	11.3	13.4	3.0	2.8	11.5	7.6	5.5	3.9	7.8	9.8	1.2	0.6	1.2	1.3	0.4	0.5		
50	18.8	19.9	14.1	15.7	3.4	3.4	10.0	7.0	4.5	3.7	7.0	9.8	0.4	0.3	0.5	0.8	0.1	0.3		

bonds when they are not in the *N*-terminal position. The symbols *x* and *y* are more convenient to use than the observed numerical values in developing the mathematical argument.

Consider now the hydrolysis of SGAGAG. The SG bond cleaves at the rate *x*, the other four bonds at the rate *z*. Thus in the small period δt hr. the number of moles hydrolysed is

$$(x + 4z) \cdot \text{SGAGAG} \cdot \delta t. \quad (2)$$

Hence the differential equation governing the rate of degradation of SGAGAG is

$$\frac{d}{dt} (\text{SGAGAG}) = -(x + 4z) \cdot \text{SGAGAG}. \quad (3)$$

When the intermediate products of hydrolysis are considered, two effects have to be noted. They are: (i) the breakdown of longer peptides is increasing the amount of the peptide under consideration; (ii) the peptide is simultaneously being hydrolysed. Thus the rate of change of quantity of the pentapeptide SGAGA, for example, is governed by the equation

$$\frac{d}{dt} (\text{SGAGA}) = z \cdot \text{SGAGAG} - (x + 3z) \cdot \text{SGAGA}. \quad (4)$$

Similarly, we have the following equations:

$$\frac{d}{dt} (\text{GAGAG}) = x \cdot \text{SGAGAG} - (y + 3z) \cdot \text{GAGAG}, \quad (5)$$

$$\frac{d}{dt} (\text{SGAG}) = z \cdot (\text{SGAGAG} + \text{SGAGA}) - (x + 2z) \cdot \text{SGAG}, \quad (6)$$

$$\frac{d}{dt} (\text{GAGA}) = x \cdot \text{SGAGA} + z \cdot \text{GAGAG} - (y + 2z) \cdot \text{GAGA}, \quad (7)$$

$$\frac{d}{dt} (\text{AGAG}) = z \cdot \text{SGAGAG} + y \cdot \text{GAGAG} - (y + 2z) \cdot \text{AGAG}, \quad (8)$$

$$\frac{d}{dt} (\text{SGA}) = z \cdot (\text{SGAGAG} + \text{SGAGA} + \text{SGAG}) - (x + z) \cdot \text{SGA}, \quad (9)$$

$$\frac{d}{dt} (\text{GAG}) = z \cdot (\text{SGAGAG} + 2 \cdot \text{GAGAG} + \text{GAGA}) + y \cdot \text{AGAG} + x \cdot \text{SGAG} - (y + z) \cdot \text{GAG}, \quad (10)$$

$$\frac{d}{dt} (\text{AGA}) = z \cdot (\text{SGAGA} + \text{AGAG}) + y \cdot \text{GAGA} - (y + z) \cdot \text{AGA}, \quad (11)$$

$$\frac{d}{dt} (\text{SG}) = z \cdot (\text{SGAGAG} + \text{SGAGA} + \text{SGAG} + \text{SGA}) - x \cdot \text{SG}, \quad (12)$$

$$\frac{d}{dt} (\text{GA}) = z \cdot (\text{SGAGA} + \text{GAGAG} + 2 \cdot \text{GAGA} + \text{GAG}) + y \cdot \text{AGA} + x \cdot \text{SGA} - y \cdot \text{GA}, \quad (13)$$

$$\frac{d}{dt} (\text{AG}) = z \cdot (\text{SGAGAG} + \text{GAGAG} + \text{SGAG} + 2 \cdot \text{AGAG} + \text{AGA}) + y \cdot \text{GAG} - y \cdot \text{AG}, \quad (14)$$

$$\frac{dS}{dt} = x \cdot (\text{SGAGAG} + \text{SGAGA} + \text{SGAG} + \text{SGA} + \text{SG}), \quad (15)$$

$$\frac{dG}{dt} = z \cdot (\text{SGAGAG} + \text{GAGAG} + \text{SGAG} + \text{AGAG} + \text{GAG}) + y \cdot (\text{GAGAG} + \text{GAGA} + \text{GAG} + \text{GA} + \text{AG}) + x \cdot \text{SG}, \quad (16)$$

$$\frac{dA}{dt} = z \cdot (\text{SGAGA} + \text{GAGA} + \text{SGA} + \text{AGA}) + y \cdot (\text{AGAG} + \text{AGA} + \text{GA} + \text{AG}). \quad (17)$$

These equations have to be integrated, if possible, into explicit formulae. Fortunately some of them yield relatively simple answers. Thus (3) immediately gives

$$\text{SGAGAG} = M e^{-(x+4z)t}, \quad (18)$$

where *M* is the number of moles of SGAGAG present at the start of hydrolysis. The other differential equations lead to more complicated formulae, and in their solution we make use of the following well-known general result. If *X* satisfies the differential equation

$$\frac{dX}{dt} = A e^{-\alpha t} + B e^{-\beta t} + \dots - \lambda X, \quad (19)$$

and *X* = 0 when *t* = 0, then explicitly,

$$X = [A/(\lambda - \alpha) e^{-\alpha t} + B/(\lambda - \beta) e^{-\beta t} + \dots] - [A/(\lambda - \alpha) + B/(\lambda - \beta) + \dots] e^{-\lambda t}. \quad (20)$$

Substituting the result (18) in (4) we find that

$$\frac{d}{dt} \text{SGAGA} = z M e^{-(x+4z)t} - (x + 3z) \cdot \text{SGAGA},$$

and applying (19) with *X* ≡ SGAGA; *A* ≡ *z**M*, $\alpha \equiv x + 4z$, $\lambda \equiv x + 3z$, we see from (20) that

$$\text{SGAGA} = M [e^{-(x+3z)t} - e^{-(x+4z)t}]. \quad (21)$$

Some of the simpler results of the integration

of the equations (4) to (17) can now be given. They are

$$\text{SGAG} = M[e^{-(x+2z)t} - e^{-(x+3z)t}], \quad (22)$$

$$\text{SGA} = M[e^{-(x+z)t} - e^{-(x+2z)t}], \quad (23)$$

$$\text{SG} = M[e^{-xt} - e^{-(x+z)t}], \quad (24)$$

$$\text{S} = M[1 - e^{-xt}], \quad (25)$$

$$\text{GAGAG} = [Mx/(x+z-y)] [e^{-(y+3z)t} - e^{-(x+4z)t}], \quad (26)$$

$$\text{GAGA} = [Mx/(x+z-y)] [e^{-(y+2z)t} - e^{-(x+3z)t} - e^{-(y+3z)t} + e^{-(x+4z)t}], \quad (27)$$

$$\text{AGAG} = M \left[\frac{z^2 + xy}{z(2z+x-y)} \cdot e^{-(y+2z)t} - \frac{xy}{z(z+x-y)} \cdot e^{-(y+3z)t} - \frac{(x+z)(z-y)}{(z+x-y)(2z+x-y)} \cdot e^{-(x+4z)t} \right]. \quad (28)$$

The other formulae are complicated and considerably more cumbersome to use in this form than those given above. Consequently, it was found better not to use the integrated equations for the remaining peptides and amino acids, but to calculate the coefficients of each exponential numerically for each value of z chosen with the observed numerical values $x=0.0056$, $y=0.0137$ and with M as 1. This was done by making use of equations (19) and (20). Thus in each equation of type (19), when x , y and z are given, the values of

$$A, \alpha, B, \beta, \dots, \lambda$$

are known. Hence by substituting these values in (20) the numerical value of each coefficient in (20) is obtained. Thus the coefficients for the tripeptides can be obtained from the known values for the tetra- and higher peptides, and similarly from these

the dipeptide coefficients can be found and, finally, those for G and A.

It should be noted from equation (25) that, as $t \rightarrow \infty$, $S \rightarrow M$. Similarly, it is obvious that, as $t \rightarrow \infty$, $A \rightarrow 2M$, $G \rightarrow 3M$. Hence the accuracy of the calculations to obtain values for A and G, in the manner described in the previous paragraph, may be checked by letting $t \rightarrow \infty$. The formulae for SGA and SG, which are fairly simple, enable a good idea of the likely value of z to be found. Further values of z around this value were then tried and the one that gave the best numerical fit was chosen. This was $z=0.0500$.

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The Effect of Growth Hormone upon Cell Division and Nucleic Acid Synthesis in the Regenerating Liver of the Rat

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Growth hormone markedly increases the low mitotic activity in the adrenal cortex of the hypophysectomized rat to levels above those found in the intact animal (Cater & Stack-Dunne, 1953). A single dose of 1 or 5 mg. of growth hormone injected into rats hypophysectomized 10–21 days previously increased the number of mitoses in the adrenal cortex in 4–8 hr. (Cater & Stack-Dunne, 1955). As a method for the study of the mode of action of growth hormone upon mitosis this preparation presents many difficulties. Holmes & Mee (1954, 1955) have reported upon nucleic acid synthesis, and

Cater, Holmes & Mee (1956) have published data on the rate of mitosis, deoxynucleic acid (DNA) and ribonucleic acid (RNA) synthesis in rat liver after partial hepatectomy. It therefore appeared of interest to investigate the effect of growth hormone upon regenerating liver. In the early stages of regeneration of liver, the various processes of the mitotic cycle can be studied separately. An increase of RNA synthesis is obvious at 11 hr. after partial hepatectomy; the rate of DNA synthesis begins to increase at about 12 hr., and at about 24 hr. after the operation is 10 times the normal rate. Mitotic activity, which is still near zero at 22½ hr., rises to 30–40/1000 cells at 27–28½ hr. after hepatectomy.

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