The Action of Trypsin on Casein

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The proteolytic enzymes from pancreas have been studied extensively ever since their discovery and several reaction mechanisms have been proposed to explain the observed kinetic data. Recently the mechanism of action of both trypsin and chymotrypsin have been shown to be more complex than was previously suggested [1,2]. Further data related to the mechanism of trypsin action are presented here.

Crystalline trypsin was kindly placed at our disposal by *Novo Terapeutisk Laboratorium*, Copenhagen, Denmark.

The substrate was a commercial »Casein nach Hammarsten». A stock solution was prepared according to Kunitz [3], except that instead of buffer the medium was 0.2 M KCl and KOH was added to adjust pH. This solution was diluted with 0.2 M KCl prior to experiment in order to give the desired concentration.

Table 1. The table gives the constants A and B. C is titration value after complete hydrolysis and can be taken as a measure of initial substrate concentration. Enzyme concentrations equals 6.5 mg/l in all experiments. (Calculations based on $\log_{10}$)

−A min	B min	C
29.2	140.0	1 080
16.4	83.4	620
8.0	44.4	340
4.75	26.1	180

The reaction was followed by continuous titration as previously described [2]. The pH was kept constant by the addition of KOH in minute quanta from a syringe; 1 titration unit equalled 0.0294 μequiv. The reaction mixture consisted of 30 ml casein solution and the reaction was initiated by the addition of 1 ml trypsin solution (20.1 mg/100 ml). Experiments were performed at pH = 7.00 and temperature = 25.3°C.

The reaction can be described by a combination of a zero order term and a first order term:

$$t = A \cdot a + B(-\log(1-a)),$$

Table 2. The table gives measured and calculated times, t_m and t_c, and degree of reaction, a. a is determined as the ratio between titration value at time t and titration value after complete hydrolysis. The calculations are based on the constants given in Table 1.

t_c	t_m	a	t_c	t_m	a	t_c	t_m	a	t_c	t_m	a
2.0	2	0.0593	1.7	2	0.0774	0.6	1	0.0471	0.8	1	0.1111
4.3	4	0.1213	3.7	4	0.1613	1.8	2	0.1412	1.8	2	0.2222
6.2	6	0.1657	6.1	6	0.2419	2.7	3	0.2000	2.8	3	0.3166
8.0	8	0.2065	8.5	8	0.3129	3.9	4	0.2706	4.0	4	0.4055
9.6	10	0.2389	9.9	10	0.3516	5.1	5	0.3324	5.1	5	0.4777
12.0	12	0.2843	12.2	12	0.4065	5.9	6	0.3676	6.0	6	0.5277
14.0	14	0.3175	14.4	14	0.4516	7.2	7	0.4205	7.4	7	0.5944
15.7	16	0.3453	16.6	16	0.4952	8.3	8	0.4617	8.4	8	0.6333
17.1	18	0.3667	18.3	18	0.5242	10.7	10	0.5412	9.3	9	0.6666
25.2	25	0.4731	20.7	20	0.5629	12.6	12	0.5941	10.0	10	0.6888
31.3	32	0.5380	25.7	25	0.6306	15.8	15	0.6647	12.6	12	0.7611
42.0	44	0.6296	30.7	30	0.6855	20.5	18	0.7471	14.2	14	0.7944
49.1	50	0.6778	35.2	35	0.7274	23.4	21	0.7853	16.0	16	0.8277
59.0	60	0.7333	41.0	40	0.7726	26.0	24	0.8147	17.5	18	0.8500
69.2	70	0.7796	46.3	45	0.8065	26.9	27	0.8235	18.7	20	0.8666
83.4	80	0.8296	55.1	55	0.8516	28.8	30	0.8412	21.8	22	0.9000
95.7	90	0.8630	60.3	65	0.8725	28.8	33	0.8412	25.4	24	0.9277
111.1	100	0.8954	68.6	75	0.9000	32.5	36	0.8706	26.3	26	0.9333

$$E \cdot t = \left(\frac{1}{k_2} - \frac{K_3}{k_1} - \frac{K_3 k_{-1}}{k_1 k_2}\right) \cdot a \cdot a - \left(\left(\frac{1}{k_1} + \frac{k_{-1}}{k_1 k_2}\right)(1 + K_3 \cdot a)\right) \log\,(1 - a)$$

where t is time, a is degree of reaction and A and B are constants. From graphs of t/a *versus* $\log\,(1-a)/a$ A and B are determined as the intercept on the ordinate and the slope, respectively. The constants A and B for four experiments with different initial concentrations of casein but with the same enzyme concentration are given in Table 1. In Table 2 the degrees of reaction are given together with measured and calculated values of t for the same four experiments.

As shown in Table 1 the constant A is negative, B is positive and both constants are proportional to the initial concentration of substrate. The tryptic action on casein thereby ressembles that on β-lactoglobulin, and the mechanism suggested for this reaction [1] could hold for the action on casein too. A mechanism involving an inhibition by the split products also could lead to a chronometric integral identical with that found empirically and so be a possible mechanism for the action of trypsin on casein and β-lactoglobulin.

The mechanism:

$$\begin{aligned} X_1 + C &\rightleftharpoons X_2 \qquad (\pm\ 1) \\ X_2 &\rightarrow X_1 + P \quad (+\ 2) \\ X_1 + P &\rightleftharpoons X_3 \qquad (\pm\ 3) \end{aligned}$$

gives the following chronometric integral by steady state treatment: (see eqn. above) which can give a negative coefficient to the zero order term and constants proportional to casein concentration if $K_3(k_2 + k_{-1}) > k_1$ and $K_3 \cdot a \gg 1$. (X_n might be an enzyme form, a combination of enzyme and substrate or a combination of enzyme and products, C is casein, P is reaction products, E is enzyme concentration, k_n is velocity constant for the n'th reaction and K_3 is the equilibrium constant for the reaction ($\pm$ 3)).

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Über die Rotationsdispersion von Tris-Diamin-Chrom(III)-Komplexen

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Bei der Messung der Absorptionsspektren von Chrom(III)-Komplexverbindungen wurde mehrfach festgestellt [1-3], dass das bei etwa 15 000 cm^{-1} ($\sim$ 650 mμ) liegende "Chromdublett", das auf Quartett-Dublett-Übergänge zurückzuführen ist [4,5], eine ausgeprägte Feinstruktur zeigt. Der Zusammenhang dieser schwachen Interkombinationsbanden mit gewissen Normalschwingungen des Moleküls [5] legte es nahe zu untersuchen, ob in diesem Spektralgebiet auch Beziehungen zwischen spektraler Absorption und Rotationsdispersion optisch aktiver Chrom(III)-Komplexe bestehen.

Für eine erste Untersuchung dieser Frage wurden die Luteochelate $[Cr\ en_3]^{3+}$ und $[Cr\ tn_3]^{3+}$ (en = Aethylendiamin, tn = Trimethylendiamin) ausgewählt, da bei ihnen die schwachen Dublettbanden noch nicht von den ersten hohen Zentralionenbanden überdeckt werden sondern frei liegen [1]. Rotationsdispersionskurven dieser beiden Verbindungen im Wellenzahlgebiet $\sim$ 17 000 – 28 000 cm^{-1} ($\sim$ 600 – 350 mμ) sind bereits seit längerem bekannt [6-8]. Mit Hilfe der in diesem Institut entwickelten Messeinrichtung [8,9] war es nun möglich,

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