versions being in this paper 0.1, 0.3, 0.6,...0.30, 0.35, 0.40, 0.45.)

In a recent work, Bader and Preston report their results from the determination of the charge density distribution in methane, not by energy minimization but by the requirement that the charge reproduces observed values of properties which are themselves functions of the one-electron charge density.[4] They obtained a charge density of 0.40 a.u. at the centers of the hydrogen atoms which is in good agreement with those reported here.

The corresponding density differences have also been recalculated whereby the molecular density is based on the "molecular" exponent 1.2 but the atomic densities to be subtracted on the "atomic" value 1.0 for the hydrogen atoms. Figs. 4, 5, and 6 show that the density difference, as expected, has maxima which now are centered on or near the sites of the hydrogen atoms.

As far as the general picture of strain is concerned, the choice of exponent for the hydrogen 1*s* orbital in the calculation of the valence electron charge density does not seem to be essential.

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Received September 12, 1970.

Activation of Chymotrypsin by Formation of a Covalent Dimer

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The activity of Chymotrypsin (CT) depends upon the existence of an ionic linkage between ile-16-NH_3^+ and asp-194-COO^- which stabilizes the active site.[1,2] The native enzyme exists as a mixture of two isomers, one of which possesses the ionic linkage and is active whereas the other lacks the ionic linkage and is inactive.[3] Binding of a specific substrate or inhibitor displaces the equilibrium towards the active form. This process, which is accompanied by rotation of the asp-194 side-chain and relief of self-inhibition was detected by optical rotatory dispersion,[4] X-ray diffraction,[3] *etc.*[1,3,5] The purpose of this work was to establish the role of the ile-16 amino group in the catalytic process by anchoring it in its charged form as an amidine. Such a modification might eliminate the conformational change and influence the activity. Removal of the charge on the amino group by acylation inactivated the enzyme whereas monofunctional amidination did not affect the activity.[5] Selective, bifunctional amidination of ile-16-NH_2 of δ-CT by malonic di-imidoester produced an inactive monomer and a dimer with strongly enhanced esterase (500—1000 fold) and reduced amidase (*ca.* 10 % left) activity. Similar, but less pronounced effects were obtained with succinic di-imidoester. The modification was performed as follows:

The amino groups of twelve times recrystallized chymotrypsinogen were exhaustively acylated with dimethylmaleic anhydride at pH 8.8—9.2 at 4°C for 10 min.[6] Excess reagent and byproducts were removed by dialysis. The zymogen was then activated by trypsin to a δ-CT which only possesses one amino group (ile 16).[7] This was amidinated by malonic di-imido ester [8] and dialyzed at pH 3 to remove byproducts and deacylate the amino groups.[13] Finally, the preparation (MCT) was fractionated on a calibrated Sephadex column. About 85 % was active dimer whereas the remainder was inactive monomer. As the amidination reagent might be sufficiently nucleophilic to displace the acyl blocks,

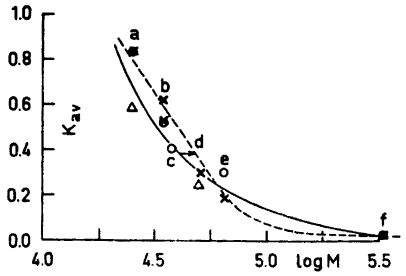

Fig. 1. Estimation of molecular weight by gel filtration on Sephadex G 100 column (56 × 2.9 cm) at 4°C and pH 7.5 in 0.05 M phosphate, saturated with $CaCl_2$ with (open symbols) and without 8 M urea (×). Markers: Chymotrypsinogen (a), pepsin (b), β-lactoglobulin (c), pepsinogen (d), hemoglobin (e), and fibrinogen (f). $K_{av}=(V_i-V_0)/(V_c-V_0)$, where V_i is the elution volume of the i-th component, V_0 the elution volume of blue dextran ($M=2\times10^6$), and V_c is the volume of the column packing. Symbols: △ MCT-monomer; ○, MCT-dimer; ——— , eluent with urea; - - - , eluent without urea. The arrow indicates the K_{av}-value of the MCT-dimer in buffer solution without urea.

a new preparation was made in which the amino groups of the zymogen were exhaustively carbamylated [9] prior to activation and amidination. The carbamyl blocks are stable and catalytic properties of MCT_{mal} and MCT_{carb} were identical.

The molecular size of the modified enzymes was determined in 0.05 M phos-

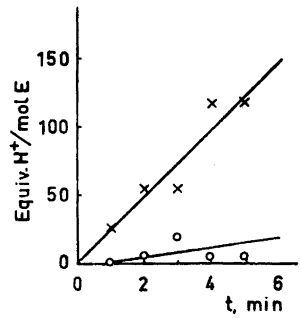

Fig. 2. Activity of α-CT (×) and MCT (○) towards *N*-acetyl-L-tyrosine amide (3.33×10^{-2} M), at pH 7.0 and 20°C in 0.004 M tris, 0.1 M $CaCl_2$. The reaction was followed by formol titration of the liberated ammonia. Ordinate: Equivalents H^+/mole enzyme.

Table 1. Structural parameters of chymotrypsin and derivatives.

	Molecular size [a]	Extinction coeff. at 280 nm l mol^{-1} cm^{-1}	Amino groups/CT-monomer [b] Found	Theory
α-CT	25 000	50 250±250	38.8±1.9	37
MCT [c]	47 000±3000	56 000±500	31.2±1.6	34
$MCT^{d}_{carb.}$	47 000±3000	56 000±500	18.2±1.4	20
S-CT [e]	—	55 300±500	—	20

[a] Determined by gel filtration on Sephadex G 100 in 0.05 M phosphate buffer with and without 8 M urea at 4°C.

[b] Includes α- and ε-amino groups and amides. Determined by ninhydrin assay using the individual extinction coefficients for NH_3, α-NH_2(ile), and ε-NH_2(lys).

[c] All free amino groups in chymotrypsinogen were masked by maleylation with dimethylmaleic anhydride prior to the activation and amidination reactions and subsequently unmasked by acid catalyzed hydrolysis.

[d] The free amino groups in chymotrypsinogen were permanently blocked by carbamylation prior to the activation and amidination reactions.

[e] Product of the treatment of α-CT with succinic di-imidoester. The procedure is analogous to the amidination of δ-CT to MCT.

Table 2. Kinetic effect of amidination, pH 8.0, 20°.

Enzyme	k_2 sec^{-1}	k_3 sec^{-1}	K_m^{app} mM	k_{cat} sec^{-1}
α-CT	3070±1250	256±175	1.1±0.3 [a]	236±16
MCT	12300±4580	542±205	0.6±0.2	519±33

$$E+S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E'S \xrightarrow{k_3} E'+P_2+P_1$$

$$K_s = \frac{k_{-1}}{k_1} = 14.2 \text{ mM}$$

S: *N*-Acetyl-L-tyr-OC_2H_5

[a] Literature value:[12] $K_m^{app}=0.7$ mM at pH 8 and 25° in 0.1 M $CaCl_2$.

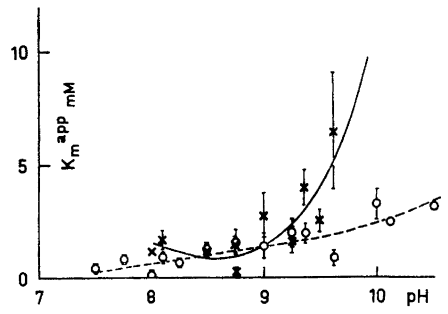

Fig. 3. The pH-dependencies of the apparent Michaelis constant for the catalysis by α-CT (×) and MCT. (○) of the hydrolysis of *N*-acetyl-L-tyrosine ethyl ester at 25°C. The vertical lines indicate the standard deviation. Lines: ———, α-CT; - - -, MCT. The curves are the simplest lines compatible with the data.

phate at pH 7.5 with and without 8 M urea on a calibrated Sephadex G 100 column (Fig. 1). Their molar extinction coefficients were determined by spectrophotometry and Kjeldahl analysis, and their content of free amino groups was found by ninhydrin assay [10] (Table 1).

The specific amidase activity [11] of MCT is illustrated in Fig. 2 and the data for specific esterolysis are listed in Table 2. In Fig. 3, a comparison between the pH-dependencies for K_m^{app} of the esterolysis of *N*-acetyl-L-tyrosine ethyl ester by α-CT and MCT is shown. It appears that the activation of the esterase activity is due to a combination of enhanced catalytic efficiency and increased affinity of the enzymes for the substrate. The displacement towards alkalinity of the p*K*-value of a group on the enzyme, which is responsible for the substrate affinity is consistent with the view that the ile-16-amino-group participates in the process of substrate binding.

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Received August 14, 1970.

Studies on Peroxomolybdates

VIII. The Structure of the Diperoxotetramolybdate(VI) Ion, $[Mo_4O_{12}(O_2)_2]^{4-}$, and of the Diperoxoheptamolybdate(VI) Ion, $[Mo_7O_{22}(O_2)_2]^{6-}$

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A proof of the existence of mono- and dinuclear peroxomolybdates has been furnished by the determination of the crystal structures of $[Zn(NH_3)_4][Mo(O_2)_4]$ and $K_2[O(MoO(O_2)_2(H_2O))_2](H_2O)_2$.[1,2] It would seem that peroxopolymolybdates also ought to exist since investigations of the crystalline products formed during the isothermal evaporation of solutions containing potassium molybdates and hydrogen peroxide in the pH range 4—8 have shown that at low H_2O_2:Mo ratios (0.1—0.8) crystals with rather large cell dimensions tend to form.[3] Judging from cell dimensions, densities, and empirical formulae, at least three different diperoxoheptamolybdates ought to exist within the pH range 4—7. The structure of one of these diperoxoheptamolybdates, $K_6[Mo_7O_{22}(O_2)_2](H_2O)_8$, has been determined and the anion is described below (I), while the structure of $K_5HMo_7O_{22}(O_2)_2(H_2O)_6$ is under consideration. For the latter compound a total of 3500 independent reflexion intensities have been collected by a PAILRED diffractometer. The crystals of $K_5HMo_7O_{22}(O_2)_2(H_2O)_6$ are monoclinic, belonging to space group $P2_1/c$, with $a=10.25$ Å, $b=17.39$ Å, $c=18.50$ Å, and $\beta=114.70°$.

In the pH range 7—8 a tetragonal peroxomolybdate with the simple empirical formula $KMoO_4$ is obtained. The structure analysis has shown that it is, in fact, a diperoxotetramolybdate with an unusual structure (see II below).

The analyses of the peroxopolymolybdates have shown that the peroxide content deviates more or less from the "ideal" value within the existence range of the phase in question. It is, however, possible to obtain a compound with the approximate stoichiometric composition, by choosing the conditions carefully. The intensity data for the diperoxoheptamolybdate were collected and processed before the comprehensive investigation of the crystalline solids separating out from solutions containing potassium molybdate and hydrogen peroxide was started, and it turned out that the structure investigation had been performed on a crystal with the composition $K_6[Mo_7O_{24-x}(O_2)_x](H_2O)_8$ where $x=1.70$. This was corrected for during the structure analysis.

The intensities of 1592 and 902 independent reflexions for I and II, respectively, were collected by the photographic Weissenberg method using Cu$K\alpha$ radiation.

The structures have been determined by the usual methods to the R values 0.109 and 0.098 for I and II, respectively.

Results. I. The crystals of $K_6[Mo_7O_{22}(O_2)_2](H_2O)_8$ are orthorhombic,[4] belonging to space group $C2cm$, with $a=8.487$ Å, $b=19.047$ Å, $c=19.974$ Å, $V=3228.8$ Å^3, and $Z=4$.

The $[Mo_7O_{22}(O_2)_2]^{6-}$ complex ion is shown in Fig. 1. It is evident that this diperoxoheptamolybdate has the same general appearance as the normal heptamolybdate,[5] the difference being that two peroxo groups replace two oxygen atoms, one at each end of the heptamolybdate. The two molybdenum atoms coordinated to the peroxo groups are thus surrounded by the pentagonal bipyramidal arrangement of ligand atoms frequently encountered in transition metal peroxo compounds. The remaining five molybdenum atoms are distortedly octahedrally coordinated by oxygen atoms as in the normal heptamolybdate.