sensitivities to Ca^{2+} inhibition; the physiological importance of the latter is therefore questionable.

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Studies on Equine Trypsinogen and Trypsin

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Equine trypsinogen was prepared by extraction of minced pancreas with 0.25 M- H_2SO_4 , fractionation with $(\text{NH}_4)_2\text{SO}_4$ and chromatography on cellulose citrate (Whatman CT-60) at pH 6.0. Inactive components were also removed from commercial bovine trypsin by this chromatographic system. Equine trypsin was prepared from trypsinogen by autocatalytic activation in the presence of 0.05 M- CaCl_2 .

Purified trypsinogen and trypsin appeared homogeneous on ultracentrifugation and by disc electrophoresis (Reisfeld, Lewis & Williams, 1962). A molecular weight of 25600 ± 1000 for equine trypsin was determined by the high-speed equilibrium method of Yphantis (1964).

The N-terminal residues of trypsinogen and trypsin were found to be serine and isoleucine respectively after carbamoylation (Stark & Smyth, 1963; Gertler & Hofmann, 1967) and these were confirmed by dansylation (Gray, 1967). Identical C-terminal sequences (Gln, Thr, Ile, Ala)-Ala-Asn were found in carboxypeptidase A digests of S-sulpho-trypsinogen and S-sulpho-trypsin.

The structure of the activation peptide was found to be Ser-Ser-Thr-Asp₄-Lys. This is similar to the activation octapeptides from pig and sheep trypsinogens but differs markedly in the N-terminal two residues of the sequence. The amino acid composition of equine trypsin showed general similarities to the trypsins from cow, pig and sheep (see Schyns, Bricteux-Gregoire & Florkin, 1969) but differed in detail in the content of methionine (1 residue/mol.) and lysine and arginine (both 7 residues/mol.).

The common features of N-terminal isoleucine and C-terminal asparagine in the trypsins from cow and pig have thus been extended to equine trypsin. Similarly, the common sequence of Asp₄-Lys reported to be in the activation peptides from cow,

pig and sheep trypsinogens has also been found in this study. The functional significance of this sequence has been reported by Abita, Delaage, Lazdunski & Savrda (1969).

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Zinc-Dependent Conformational Stability in the Alkaline Phosphatase of *Escherichia coli*

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Alkaline phosphatase of *Escherichia coli* has been found variously to contain 4, 3 or 2 g.atoms of Zn/mole (Simpson & Vallee, 1968; Reynolds & Schlesinger, 1967; Plocke, Levinthal & Vallee, 1962); at least part of the zinc content is essential to activity, and its absence through chelation or genetic alteration (Schlesinger, 1966) renders the enzyme inactive. Zinc has also been shown (Reynolds & Schlesinger, 1967) to be essential for subunit re-association but not necessary for refolding of the subunit from an extended-coil conformation.

In the present work ultraviolet circular dichroism (CD) has been employed to detect major conformational phenomena related to the presence of zinc, with wild-type enzyme from *E. coli* K 12 and a zinc deficient enzyme from *E. coli* C₁₀F₁₈.

The CD extrema near 221 and 207 nm. remained essentially unchanged when *E. coli* wild-type enzyme was suspended in high urea concentrations; the 192 nm. peak became inaccessible. *E. coli* wild-type enzyme depleted of zinc by dialysis against EDTA behaved quite differently; substantial deviation from natural conformation occurred at above 2 M-urea, characterized by loss of the 221 nm. and 207 nm. peaks and the appearance of a larger peak near 202 nm. Sedimentation-velocity analysis of EDTA-treated enzyme in 6 M-urea revealed that dissociation into subunits had occurred; the low sedimentation coefficient, $S_{20,w}$ 1.7s, was consistent with an extremely extended conformation.

E. coli C₁₀F₁₈ inactive enzyme behaved similarly to EDTA-treated *E. coli* wild-type enzyme when treated with urea solutions. *E. coli* C₁₀F₁₈ enzyme incubated with Zn^{2+} and phosphate with partial

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