CHEMICAL EVIDENCE FOR THE INVOLVEMENT OF TRYPTOPHAN IN THE INTERACTION OF TRYPSIN WITH THE INHIBITOR FROM BEEF PANCREAS

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We have recently shown that pH is an important parameter for the selective oxidations of proteins by N-bromosuccinimide (Green and Witkop, 1964). An application of this technique is described in the following.

The environment of the tryptophan residues of trypsin is modified on interaction with several naturally occurring trypsin inhibitors, among them the inhibitor from beef pancreas (PTI), a tryptophan-free, basic protein of MW 6,500 (Laskowski and Laskowski, 1954). The evidence for this observation rests on spectral methods (Edelhoch and Steiner, 1965).

We now wish to present chemical evidence which shows that tryptophan in the trypsin-PTI complex is protected from NBS, the tryptophan-specific oxidant (Witkop, 1961), and that pH has a remarkable effect on these oxidations, as it also has on the hydrogen-deuterium exchange rates of proteins (Hvidt, 1964).

At pH 4, nearly all the four tryptophan residues in trypsin are oxidized by NBS in a rapid and specific react-

Table.—The Selective Modification of Tryptophan Residues in Trypsin and Trypsin-PTI Complex by NBS

(0.1 M Acetate: 25.0°)

pH	7.0	6.0	5.5	5.0	4.5	4.0	3.5	3.0
			Trypsin (conc. = 2.17 mg/2.0 ml)					
Extent of Oxidation (%)[b]	13	25	44	65	80	90	93	96
Moles NBS added / Mole Try oxidized	11	8.7	7.2	4.2	3.5	3.1	3.0	2.6
			Trypsin-PTI Complex (1:1 conc. = 2.08 mg trypsin[d]/2.0 ml)					
Extent of Oxidation (%)	--	4.1[a]	5.3 6.5[a]	16 55[c]	44	58	65	94 103[c]
Moles NBS added / Mole Try oxidized	--	13	15	8.6	3.1	2.8	2.9	2.9 2.1[c]

a) 0.1 M phosphate.

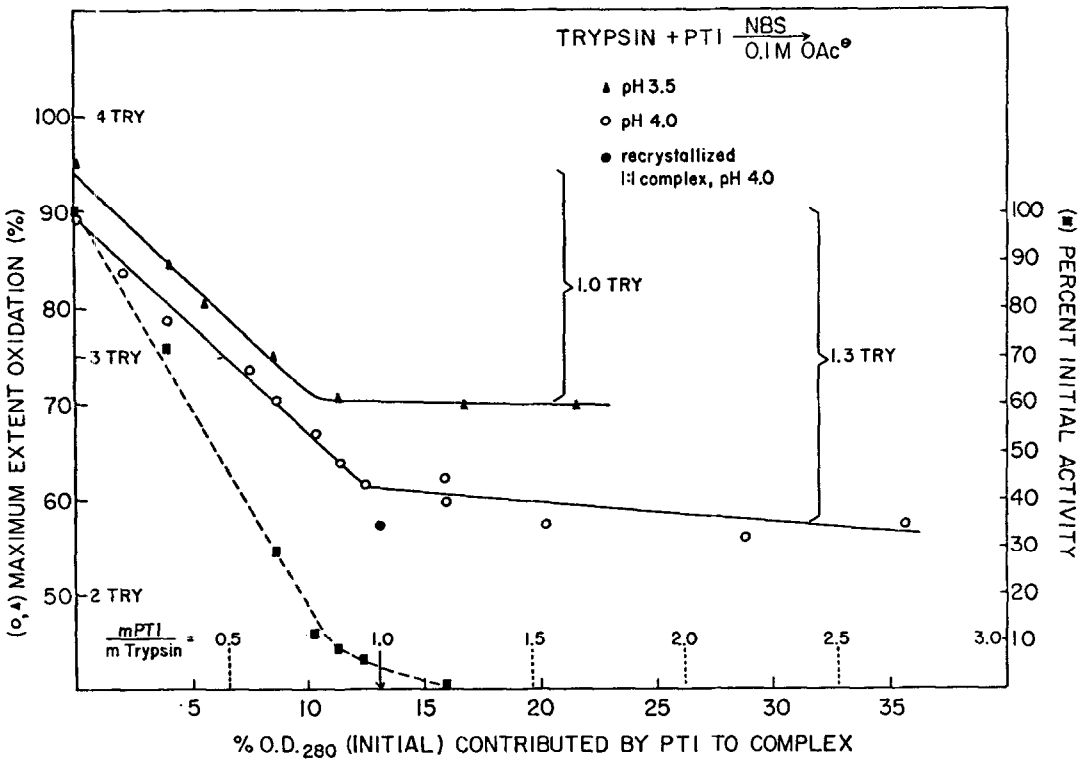
b) The extent of oxidation $\frac{\text{moles tryptophan oxidized}}{\text{moles tryptophan present}} \times 100$ can be derived from the simple expression: % tryptophan oxidation = % decrease $O.D._{280} \times 2.02$; the empirical factor 2.02 is characteristic of trypsin and used only for this protein.

c) 8 M Urea adjusted to the desired pH with HOAc.

d) The factor 0.869 is the resultant of the optical factors at 280 mμ of trypsin (0.695) and of PTI (1.26) (Laskowski and Laskowski, 1954); it was used to correct the optical density of the complex for the contribution due to trypsin alone.

ion (Viswanatha et al., 1960). As the pH of the oxidation medium is raised, oxidation of tryptophan becomes increasingly more difficult (Green and Witkop, 1964).

When the 1:1 complex of trypsin with PTI (Worthington Biochemical Corp.) is titrated (acetate buffer) with a dilute aqueous solution (10 mM) of NBS and the course of the oxidation is followed by the decrease in optical density at 280 mμ, (Patchornik et al., 1958; and Spande and Witkop, 1965) significant differences in the maximum extent of oxidation, possible at a given pH, are noted in comparison with the oxidation of native trypsin under the same conditions (Table). The protection of tryptophan residues in the trypsin-PTI complex is especially noticeable at pH



values >3.0. The maximum difference in the oxidation is observed at pH 5 where it approaches two tryptophan equivalents.

If PTI is added in increments to a solution of trypsin at either pH 3.5 or 4.0 and the oxidation with NBS carried out as above, a linear decrease in the percent of oxidizable tryptophan in the trypsin sample is observed up to the point of addition of one equivalent of the inhibitor (Fig. 1). Further increments have little effect. At pH 3.5 one residue, at pH 4.0 approximately 1.3 residues of tryptophan are protected from the action of NBS. Trypsin after incubation at pH 5.5 with a large excess of the trypsin substrate p-toluene sulfonyl-L-arginine methyl ester and rapid titration with NBS at pH 3.5 or 4.0 shows no detectable difference from native trypsin. It is therefore assumed that in the trypsin-PTI complex some tryptophan residues are directly involved in binding.

The trypsin-PTI complex dissociates in part at acidic pH values. However, activity measurements at pH 4.0 suggest that the complex is not appreciably dissociated at this pH.

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