

## DETERMINING THE PRIMARY STRUCTURE

### Chemical analysis

① 2, 4 - dinitrofluorobenzene (DNFB) / Sanger's rgt  
 ↗ dinitrophenol  
 DNP - aa - aa - aa  
 { hydrolyze 6M HCl

DNP - aa      aa      aa  
 ↘  
 N-terminal aa

② phenylisothiocyanate / Edman's rgt  
 ↗ phenylthiohydantoin  
 PTH - aa - aa - aa

↓  
 PTH - aa      aa - aa  
 ↘  
 PTH - aa - aa  
 ↓  
 PTH - aa      aa

sequential  
 clearing of  
 N-terminal aa

### ③ Hydrazine

aa - aa - aa - faa  
 ↘  
 C-terminal aa

### ④ Cyanogen Bromide (CNBr)

aa - aa - aa - Met - faa - aa

### ⑤ Hydroxylamine HCl

aa - aa - aa - Nsn - f Gly - aa - aa

### Enzymatic Hydrolysis

#### ① Trypsin

aa - aa - Lys - f aa - aa

aa - aa - Arg - f aa - aa

#### ② chymotrypsin

aa - aa - Tyr - f aa - aa

aa - aa - Phe - f aa - aa

aa - aa - Trp - f aa - aa

#### ③ carboxypeptidase A

aa - aa - aa - f aa  
 ↘  
 all C-terminal aa  
 EXCEPT Pro

#### ④ carboxypeptidase B

aa - aa - aa - f Lys } C-terminal  
 aa - aa - aa - f Arg } only

#### ⑤ Thermolysin

aa - aa - aa - f Ile - aa

aa - aa - aa - f Val - aa

aa - aa - aa - f Leu - aa

#### ⑥ streptococcal protease

aa - aa - aa - Asp - f aa

aa - aa - aa - Glu - f aa