

The last problem in determining the complete covalent structure of a protein is to elucidate the positions of the disulfide bonds within and between polypeptide chains.

Consider that N- and C-terminal analysis of a protein indicated the presence of two polypeptide chains and that treatment of the protein with NaBH_4 followed by $\text{ICH}_2\text{CO}_2^-$, separation and sequence determination gave

$\text{Ala}_1\text{-Val}_2\text{-Cys}_3\text{-Gly}_4\text{-Ser}_5\text{-Lys}_6\text{-Cys}_7\text{-Glu}_8$

$\text{Gly}_1\text{-Cys}_2\text{-Val}_3\text{-Arg}_4\text{-Thr}_5\text{-Cys}_6\text{-Glu}_7$

Note that there are two possible disulfide bonding arrangements with two disulfide bonds between chains.

Cys₃ in the first chain may form a disulfide bond to Cys₂ or Cys₆ in the second chain and correspondingly Cys₇ in the first chain may form a disulfide bond to Cys₆ or Cys₂ in the second chain.

To determine which arrangement is present it is necessary to treat a sample of the protein with CNBr or an endopeptidase to obtain fragments with the disulfide bonds intact.

If the two chains are cleaved between the cys residues, one can determine which cys residues form the disulfide bonds.

Based on the sequence of the two chains, treatment of the protein with trypsin would give two fragments (A and B) each of which would contain a shorter peptide from one chain joined by a disulfide bond to a shorter peptide from the other chain.

Consider that separation of the fragments from each other by electrophoresis or chromatography followed by treatment with NaBH_4 and $\text{ICH}_2\text{CO}_2^-$ to separate the shorter peptides, and sequence determination of the two peptides from each fragment gave

Fragment A

Ala-Val-Cys-Gly-Ser-Lys

Thr-Cys-Glu

Fragment B

Cys-Glu

Gly-Cys-Val-Arg

The results indicate that Cys₃ in the first chain forms a disulfide bond to Cys₆ in the second chain and that Cys₇ in the first chain forms a disulfide bond to Cys₂ in the second chain as follows

Ala₁-Val₂-Cys₃-Gly₄-Ser₅-Lys₆-Cys₇-Glu₈

Gly₁-Cys₂-Val₃-Arg₄-Thr₅-Cys₆-Glu₇

Let's begin to describe the shapes that polypeptide chains conform to in aqueous solution.

First consider the bond angles and bond distances associated with the atoms forming the peptide bond as shown in the handout figure.

Bond angles of the α - carbon atoms ($\sim 109^\circ$) correspond to sp^3 hybrid orbitals.

The 3 σ bonds associated with each of the carbonyl C and N atoms involve sp^2 hybrid orbitals whose axes are planar and form angles of $\sim 120^\circ$.

The C-N peptide bond distance (1.325 \AA) is intermediate between C-N single (1.487 \AA) and double (1.27 \AA) bonds.

The shorter bond distance is due to the following resonance structures:

The handout shows the molecular orbitals corresponding to each of these resonance forms.

The actual structure corresponds to a hybrid of the two resonance forms with the molecular orbital description shown in the handout.

It has been calculated that the second resonance structure stabilizes the peptide bond by 21 kcal/mole.