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PEPTIDE SEQUENCING CHALLENGE

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Meet the challenge

While on a Red Cross relief effort in the Congo region in 1960s a Norwegian doctor noted that African women often ingested a tea made from leaves of a local plant to assist the childbirth. The active ingredient, named after the local name for native medicine, was isolated a decade later and was a small thirty amino acid peptide. It further took some 25 years to fully elucidate the structure of this compound.

The challenge

While trying to determine the primary structure of this peptide, it was noticed that the classical Edman degradation method failed to give structural information. Thus, several chemical cleavage and degradation methods had to be combined together in order to obtain the sequence information. The primary structure of this protein was finally obtained in the mid 1990s and comprised a rare phenomenon among the plant peptides – the peptide was cyclic.

This cyclic peptide has six cysteine moieties, which together form three disulfide bridges. To determine the connectivity of the cysteines, the following experiment was carried out. First, the peptide was partially reduced with $(\text{HOOC-CH}_2\text{CH}_2)_3\text{P}$ at low pH, and the reduced $-\text{SH}$ groups were then alkylated with *N*-ethylmaleimide. Then, complete reduction of the remaining S-S groups was performed with dithiothreitol, and the resulting $-\text{SH}$ groups were ethylated with

bromoethylamine. The obtained peptides were separated using chromatography and then cleaved with trypsin for massspectrometry analysis.

Four peptides were obtained from the partial reduction experiment: the native peptide, the fully-reduced peptide, and two partially-reduced peptides. Tryptic fragments of the two partially reduced peptides are shown in Table 1, where an asterix denotes the maleimide ethylation and a double asterix – the aminoethylation. In addition, the structure of the native cyclic peptide is represented as the following string:

Table 1.

Tryptic fragments of the two partially reduced peptides

Fragment	First peptide	Second peptide
1	{3}Cys*{4}Cys*{5}Cys**	{3}Cys**
2	{7}Cys*{1}Cys*{2}Cys**	{4}Cys*{5}Cys**
3	{3}Cys*{4}Cys*{5}Cys**{6}	{7}Cys**{1}Cys*{2}Cys**
4	{6}{7}Cys*{1}Cys*{2}Cys**	{7}Cys**{1}Cys*{2}Cys**{3}Cys**

The challenge

Determine the disulfide bonding in the analyzed peptide. *What is special about the disulfide connectivity that decorates this peptide?*