

Experimental methods in protein sequence analysis

Edman sequencing was performed on the isolated peptide fragments of the protein. The peptides that were sequenced correspond to their tryptic peptide numbers in the tryptic map above. PTH-amino acids that are in parentheses indicate ambiguous amino acid assignments; the yields of those PTH-amino acids were very low and likely were at the level of detection as the background peaks.

Microsequence analysis of tryptic peptides

Cycle	Amino acid (pmol)													
	T2	T3	T6a	T6b	T6c	T8	T14	T15	T18	T19	T23	T24	T25	
1	Cys (227)	Ala (329)	Cys (86)	Lys (111)	Val (313)	Gly (221)	Thr (100)	Ile (543)	Ser (103)	Ser (36)	Glu (184)	Ser (194)	Leu (170)	
2	Asn (262)	Glu (275)	Asn (132)	Val (238)	Ile (300)	Leu (197)	Pro (151)	Thr (111)	Ile (62)	Ile (68)	Pro (122)	Val (416)	Ser (84)	
3	Asn (246)	Glu (380)	Asn (159)	Ile (273)	Phe (289)	Cys (294)	Glu (264)	Gly (876)	Gly (159)	Gly (81)	Asp (197)	His (192)	Pro (149)	
4	—	His (84)	—	Phe (203)	Cys (194)	Leu (192)	Asp (122)	Leu (948)	Ile (38)	Ile (81)	Ser (68)	Leu (270)	Asp (119)	
5	Gly (122)	Tyr (203)	Gly (84)	Cys (140)	Ser (122)	Ser (151)	Thr (73)	Asp (1080)	Glu (184)	Glu (111)	Asn (262)	Phe (535)	Asp (173)	
6	Tyr (138)	Pro (125)	Tyr (95)	Ser (39)	(Arg) (22)	Cys (159)	Ala (248)	Pro (764)	Lys (127)	Lys (108)	Val (103)	Ile (297)	Ala (138)	
7	Glu (122)	Val (289)	Glu (79)	Arg (14)	—	Arg (51)	Glu (122)	Ala (659)	Pro (135)	Pro (73)	Ile (70)	Asp (286)	Asp (138)	
8	Ile (122)	Ser (167)	Ile (59)	—	—	—	Asp (78)	Gly (386)	Val (97)	Val (143)	Val (68)	Ser (97)	Phe (59)	
9	Asn (103)	Ala (148)	Asn (76)	—	—	—	Asp (103)	Pro (348)	Gly (135)	Gly (70)	Val (81)	Leu (186)	Val (84)	
10	Lys (20)	Gly (86)	Lys (22)	—	—	—	Cys (303)	Asn (564)	His (154)	His (43)	Asp (130)	Leu (394)	Asp (97)	
11	—	Tyr (103)	—	—	—	—	(His) (23)	Phe (370)	Val (100)	Val (149)	Trp (89)	Asn (265)	Val (111)	
12	—	(Thr)	—	—	—	—	Leu (57)	Glu (446)	Asp (176)	Asp (81)	Leu (65)	Glu (157)	Leu (95)	
13	—	(Lys)	—	—	—	—	Ile (38)	Tyr (356)	Ile (49)	Ile (73)	Ser (14)	Glu (132)	His (59)	
14	—	—	—	—	—	—	Pro (86)	Ala (389)	Tyr (132)	Tyr (51)	Tyr (22)	Asn (159)	Thr (24)	
15	—	—	—	—	—	—	Gly (46)	Glu (324)	Pro (105)	Pro (35)	(Lys) (22)	Pro (103)	Phe (46)	
16	—	—	—	—	—	—	(Val)	Ala (346)	Asn (24)	Asn (35)	—	Ser (16)	(Thr)	
17	—	—	—	—	—	—	(Val)	Pro (219)	Gly (14)	Gly (24)	—	(Lys) (65)	(Arg)	
18	—	—	—	—	—	—	(Glu)	(Ser)	(Gly)	(Gly)	—	—	—	
19	—	—	—	—	—	—	—	(Arg)	—	—	—	—	—	

4. Peptide T24 has an amino-terminal Ser (serine) residue as indicated by the detection of PTH-Ser as the first cycle of Edman sequencing of that peptide. Why is the yield of the PTH-Ser lower than the succeeding PTH-amino acids identified in the sequence?

5. In the actual protein, peptide T15 C-terminal Arg is in a peptide bond with the N-terminal Leu of peptide T25. The two peptides are contiguous such that the full-length sequence of T15-T25 would show an N-terminal Ile (isoleucine) amino acid and a C-terminal Arg (arginine) amino acid. This alignment of these two tryptic peptides, T15 and T25, were deduced from sequence homology to another similar protein whose sequence was already known.

If sequence homology data were not available, how might you experimentally determine the alignment of peptides T15 and T25 that reveals this contiguous (T15-T25) amino acid sequence? [Remember that Edman sequencing of the intact protein could only identify residues 1 through residue 19 so one cannot perform Edman amino acid sequence determination on the full-length protein.]

Methods In Protein Sequence Analysis Experimental Biology Medicine Ser Vol 3

Xiaolong Qi



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