Mass spectrometry evaluation of type I Collagen in-situ digestion in biological matrices

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Introduction:

Type I collagen crosslinked telopeptide (β-CTX) is a biomarker commonly used to monitor compliance of osteoporotic patients to antiresorptive therapy. It is currently used by an algorithm recommended by the IOF and the IFCC.[1] Three immunoassays are currently commercially available for the quantitation of β-CTX.[2] However, plasma and serum samples collected from the same patients do not provide the same concentration and do not degrade at the same rate. As β-CTX is an uncharacterized fragment of the C-terminal telopeptide of type I collagen, it was decided to study the in-situ digestion of type I collagen in plasma and serum in order to explain the differences between both matrices.

Materials and Methods:

One pool of EDTA plasma and one pool of serum were prepared with remnant samples from hemodialyzed patients in order to obtain high contractions of peptides. Pools were precipitated with zinc sulphate and acetonitrile, evaporated to dryness and reconstituted with 1 mL of injection solvent. Each reconstituted pools was then analysed using a LC-HDMSe (SYNAPT XS, Waters) workflow. PLGS software was then used to identify the fragments belonging to type I collagen.

Results:

Sequences, m/z ratios and the locations of the identified peptides are reported in Table 1. Fragments from the different regions of the type I collagen (namely the telopeptides and the helix) were identified in both matrices. Fourteen peptides were found in EDTA plasma while twenty-nine were identified in serum. This difference could be explained by the fact that divalent ions such as Ca^{2+} and Zn^{2+} are chelated in EDTA plasma thus preventing proteolytic enzymes present in blood to cleave the type I collagen.

Perspectives:

Further purification steps will be considered in order to identify more peptides from type I collagen in both matrices by decreasing ion suppression triggered by highly concentrated peptides. Purification by preparative liquid chromatography and digestion.

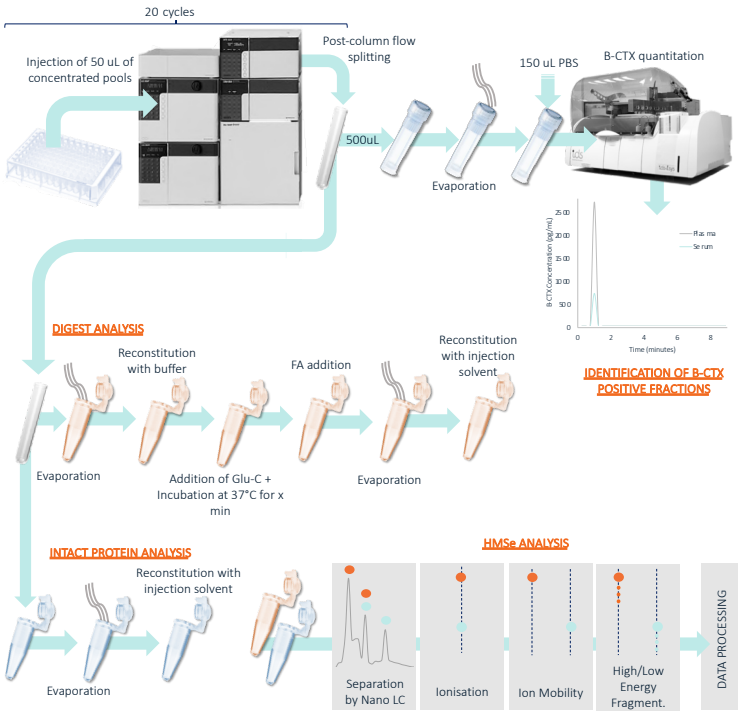

Table 1. Identified type I collagen peptides

SERUM

		SEQUENCE	m/z
13	43	(L)LAATALLTHGQEEGQVEGQDEDIPPITCVQN(G)	3276,5706
23	73	(G)QEEGQVEGQDEDIPPITCVQNGLRYHDRDVWKPEPCRICVCDNGKVLCDDVI(C)	5925,731
122	137	(G)PRGPAGPPGRDGIPGQ(P)	1528,797
128	137	(G)PPGRDGIPGQ(P)	993,513
167	177	(G)YDEKSTGGISV(P)	1155,5514
191	208	(P)GPPGAPGPQGFQGPPGEP(G)	1643,7913
214	276	(R)PGERGPPGPQGARGLPGTAGLPGMKGHRGFSGLDGA(K)	3409,6982
288	326	(G)EPGSPGENGAPGQMGPRGLPGERGRPGAPGPAGARGNDG(A)	3634,7104
364	380	(S)EGPQGVRGEPGPPGPAG(A)	1558,7632
581	593	(M)GFPGPKGAAGEPG(K)	1141,5626
683	738	(P)GERGVQGPPGPAGPRGANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGERGAAGL(P)	4942,369
684	691	(G)ERGVQGPP(G)	8394,363
747	792	(G)DAGPKGADGSPGKDGVRGLTGPIGPPGPAGAPGDKGESGPSGPAGP(T)	4016,9453
767	806	(T)GPIGPPGPAGAPGDKGESGPSGPAGPTGARGAPGDRGEPG(P)	3459,6477
769	793	(P)IGPPGPAGAPGDKGESGPSGPAGPT(G)	2128,0298
775	780	(P)AGAPGD(K)	4872,076
801	815	(G)DRGEPGPPGPAGFAG(P)	1381,6501
834	892	(G)AKGDAGPPGPAGPAGPPGPIGNVGAPGAKGARGSAGPPGATGFPGAAGRVGPPGPSGNA(G)	4958,6064
927	964	(G)PPGPAGEKGSPGADGPAGAPGTPGPQGIAGQRGVVGLP(G)	3328,7595
932	948	(A)GEKGSPGADGPAGAPGT(P)	1425,6597
939	966	(G)ADGPAGAPGTPGPQGIAGQRGVVGLPGQ(R)	2482,2786
974	980	(P)GLPGPSG(E)	5843,052
1001	1006	(M)GPPGLA(G)	511,2875
1103	1121	(Q)GDRGIKGHRGFSGLQGPPG(P)	18929,865
1115	1136	(S)GLQGPPGPPGSPGEQGPSGASG(P)	18878,771
1188	1210	(G)PPGPPSAGFDFSFLPQPPQEKAH(D)	24481,975
1317	1328	(K)NPKDKRHVWFGE(S)	1512,77
1397	1432	(I)EIRAEGNSRFTYSVTVDGCTSHTGAWGKTVIEYKTT(K)	3964,916
1451	1456	(P)DQEFGF(D)	7423,067

PLASMA

23	32	(G)QEEGQVEGQD(E)	1118,4581
89	105	(E)GECCPVCPDGSESPTDQE(T)	1852,6678
194	237	(P)GAPGPQGFQGPPGEPGEPGASGPMGPRGPPGPPGKNGDDGEAGK(P)	4016,8591
213	246	(G)ASGPMGPRGPPGPPGKNGDDGEAGKPGRPGERGP(P)	3222,5452
249	274	(G)PQGARGLPGTAGLPGMKGHRGFSGLD(G)	2534,3047
279	319	(G)DAGPAGPKGEPGSPGENGAPGQMGPRGLPGERGRPGAPGPA(G)	3773,8242
438	448	(G)APGSKGDTGAK(G)	988,4906
443	495	(K)GDTGAKGEPGPVGVQGPPGPAGEEGKRGARGEPGPTGLPGPPGERGPGSRGF(P)	4899,4263
721	734	(S)QGAPGLQGMPGERG(A)	1354,6515
791	829	(A)GPTGARGAPGDRGEPGPPGPAGFAGPPGADGQPGAKGEP(G)	4016,8591
995	1000	(R)GPPGPM(G)	571,255
1204	1217	(Q)PPQEKAHDGGRYYR(A)	1673,8156
1220	1231	(D)DANVVRDRDLEV(D)	1400,716
1335	1347	(F)QFEYGGQGSDPAD(V)	1370,5485

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