RESULTS

1. RAGE human is the only protein identified in samples A and B

Samples A and B (1 µg each) were digested in solution with trypsin and the peptide mixtures obtained were analysed by LC-MS/MS. Data processing and searching were performed using VEMS_3 [Matthiesen *et al.,* (2005). J. Proteome. Res. 4, 2338-2347] against Uniprot-SwissProt database. Only RAGE_HUMAN (Q15109) was identified in both samples in addition to trypsin and keratins.

2. Fragments of human RAGE are present in samples A and B

Mass determination of intact proteins in samples A and B (1 µg each) was carried out by LC-MS using a C4 RP column. The major component of sample A has a mass of 33165.9 Da and sample B has three major components of 33164.6, 24581.8 and 12520.4 Da. Sample A also contained some minor components that are described in section 5. The best way to match these masses with fragments of RAGE is shown in the table. This assignment was facilitated by the fact that recombinant proteins expressed in *E. coli* do not usually contain the signal sequence.

	N-terminal sequence	C-terminal sequence	Observed mass	Expected mass(disulfide bridges oxidised)
RAGE_A1	GSHM^{23}AQN	IEPGEEG^{328}	33165.9	33162.9
RAGE_B1	GSHM^{23}AQN	IEPGEEG^{328}	33164.6	33162.9
RAGE_B2	GSHM^{23}AQN	EVQLVVE^{243}	24581.8	24581.2
RAGE_B3	GSHM^{23}AQN	IVDSASE^{132}	12520.4	12522.4

3. Peptides from N- and C-terminal sequences of the RAGE fragments are identified

Sample A and B were separately digested with trypsin, chymotrypsin and Glu-C. Peptide mixtures were analysed by LC-MS/MS and the processed data were used to search in a home-made database where sequences of different human RAGE fragments and the isoforms and sequence variants described in RAGE_HUMAN Uniprot entry (Q15109) were added. This experiment allowed us to identify the following N- and C-terminal sequences.

Sample		Trypsin	Chymotrypsin	GluC
A	N-terminal	GSHM^{23}AQNITAR	GSHM^{23}AQNITARIGEPL	
	C-terminal		SCVATHSSHGPQESRAVSI SIIEPGEEG^{328}	SRAVSISIIEPGEEG^{328}
B	N-terminal	GSHM^{23}AQNITAR	GSHM^{23}AQNITARIGEPL	
	C-terminal	VRVYQIPGKPEI VDSASE^{132}		
			RTAPIQPRVWEPVPLEEVQ LVVE^{243}	
			SCVATHSSHGPQESRAVSI SIIEPGEEG^{328}	SRAVSISIIEPGEEG^{328}

The identification of these peptides is only possible if they are the N- and C-terminal sequences, because one of their ends is not protease specific.
Therefore these results confirmed the presence of the fragment RAGE_A1 in sample A, and the fragments RAGE_B1, RAGE_B2 and RAGE_B3 in sample B.

4. A sequence coverage of 100% is obtained and no sequence variants of RAGE are identified

Results of the searches described in 3 were merged in order to get the maximum sequence coverage for each of the RAGE fragments identified. In sample A 100% sequence coverage was obtained for RAGE_A1 fragment. In sample B 100% sequence coverage was obtained for RAGE_B2 and RAGE_B3 fragments and 99% for RAGE_B1. Specific peptides to the isoform 2 or sequence variants described in human RAGE Uniprot entry (Q15109) were not identified.

5. Small amounts of multiple fragments of human RAGE are present in sample A
The mass analysis of intact proteins described in section 2 also showed the presence of the following fragments as minor components (all together less that 5%) in sample A.

Observed mass	Expected mass (disulfide brigdes oxidised)	N-terminal sequence	C-terminal sequence
21438.3	21436.5	GSHM^{23}AQN	CSFSPGLPR^{216}
21732.0	21729.9	GSHM^{23}AQN	FSPGLPRHR^{218}
21802.2	21800.9	GSHM^{23}AQN	SPGLPRHRA^{219}
22836.9	22834.2	GSHM^{23}AQN	LRTAPIQPR^{228}
22934.4	22933.3	GSHM^{23}AQN	RTAPIQPRV^{229}
24014.9	24012.5	GSHM^{23}AQN	WEPVPLEEV^{238}
24143.0	24140.7	GSHM^{23}AQN	EPVPLEEVQ^{239}
24256.2	24253.8	GSHM^{23}AQN	PVPLEEVQL^{240}
24355.4	24353.0	GSHM^{23}AQN	VPLEEVQLV^{241}
24583.2	24581.2	GSHM^{23}AQN	LEEVQLVVE^{243}
24808.6	24807.4	GSHM^{23}AQN	EVQLVVEPE^{245}
24868.5	24864.5	GSHM^{23}AQN	VQLVVEPEG^{246}
25319.6	25317.0	GSHM^{23}AQN	PEGGAVAPG^{252}
25376.4	25374.0	GSHM^{23}AQN	EGGAVAPGG^{253}
25680.2	25675.4	GSHM^{23}AQN	AVAPGGTVT^{256}
25792.1	25788.5	GSHM^{23}AQN	VAPGGTVTL^{257}
25891.7	25889.7	GSHM^{23}AQN	APGGTVTLT^{258}

As these fragments were present in small amounts, we have not considered them as major components of the sample and, therefore, we have not included them in the survey. Whether these minor components have been added to the sample on purpose or are either contaminants or degradation products due to sample handling is something we have not been able to elucidate.