**Abstract**

The presence of proteolytic inhibitors in sera or plasma samples has been found to significantly affect the outcome profile of selected plasma proteins as seen on two-dimensional electrophoresis (2-DE) gels. With the inclusion of a protease inhibitor cocktail, many human plasma protein isoforms were revealed, which could be further analyzed by MALDI-TOF mass spectrometry. The inhibition of endogenous proteolytic activity in the intact sample via the use of a protease inhibitor cocktail provides a significant advantage in the detection of protein isoforms. The characterization of the Apo A1 isoform train was accomplished by MALDI-TOF MS evaluation of peptides derived from each of the isoforms present on 2-DE gels. Table 1 gives a comparative tryptic peptide list for each isoform. Four of the nine Apo A1 peptides identified were found to have a partner peptide with a molecular weight increase of 0.93 Da, suggesting the occurrence of specific proteolysis within the protease inhibitor cocktail (see Table 1). The spots in this train have molecular masses and pI's similar to Apolipoprotein A2.

**Introduction**

Protein isoforms are commonly used as a protein analysis system to profile proteins from endogenous and exogenous sources. In this study, we used a protease inhibitor cocktail to analyze the plasma protein profile of human plasma samples prepared by Western blotting (1) and MALDI-ToF MS (2). The depletion of total plasma samples (9) was achieved by IgG depletion. The depleted samples (100 µg for Panels A and B, and 200 µg for Panel C) were separated on 2-DE gels as described in the Methods section. Panels D, E, and F are magnifications of the Apo A1 train from gels A, B, and C respectively.

**Methods**

**Protein Mass Spectrometric Analysis**

Plasma samples (HAPPY PRP III, 90µL of 20 or 100µL) were thawed and then spiked with internal standards. The samples were then spotted on a MALDI target and allowed to dry at room temperature before introduction into the mass spectrometer. The depleted samples (100 µg for Panels A and B, and 200 µg for Panel C) were separated on 2-DE gels as described in the Methods section. Panels D, E, and F are magnifications of the Apo A1 train from gels A, B, and C respectively. With the inclusion of a protease inhibitor cocktail, many human plasma protein isoforms were revealed, which could be further analyzed by MALDI-TOF mass spectrometry. The inhibition of endogenous proteolytic activity in the intact sample via the use of a protease inhibitor cocktail provides a significant advantage in the detection of protein isoforms. The characterization of the Apo A1 isoform train was accomplished by MALDI-TOF MS evaluation of peptides derived from each of the isoforms present on 2-DE gels. Table 1 gives a comparative tryptic peptide list for each isoform. Four of the nine Apo A1 peptides identified were found to have a partner peptide with a molecular weight increase of 0.93 Da, suggesting the occurrence of specific proteolysis within the protease inhibitor cocktail (see Table 1). The spots in this train have molecular masses and pI's similar to Apolipoprotein A2.

**Western Blotting**

Western blotting (13) of plasma samples (9) were isolated and blotted using PAb(Apo A1) and PAb(Apolipoprotein A2) as detected by the antibodies APOBEC-1 and APOBEC-2, respectively. The depleted samples (100 µg for Panels A and B, and 200 µg for Panel C) were separated on 2-DE gels as described in the Methods section. Panels D, E, and F are magnifications of the Apo A1 train from gels A, B, and C respectively. The depleted samples (100 µg for Panels A and B, and 200 µg for Panel C) were separated on 2-DE gels as described in the Methods section. Panels D, E, and F are magnifications of the Apo A1 train from gels A, B, and C respectively. With the inclusion of a protease inhibitor cocktail, many human plasma protein isoforms were revealed, which could be further analyzed by MALDI-TOF mass spectrometry. The inhibition of endogenous proteolytic activity in the intact sample via the use of a protease inhibitor cocktail provides a significant advantage in the detection of protein isoforms. The characterization of the Apo A1 isoform train was accomplished by MALDI-TOF MS evaluation of peptides derived from each of the isoforms present on 2-DE gels. Table 1 gives a comparative tryptic peptide list for each isoform. Four of the nine Apo A1 peptides identified were found to have a partner peptide with a molecular weight increase of 0.93 Da, suggesting the occurrence of specific proteolysis within the protease inhibitor cocktail (see Table 1). The spots in this train have molecular masses and pI's similar to Apolipoprotein A2.

**Gel Analysis and In-Gel Digestion**

Stained gels were imaged and evaluated using the Phoretix 2-DS imaging software (Phoretix Ltd, Newcastle Upon Tyne, UK). The Phoretix gel analysis software (Phoretix Ltd, Newcastle Upon Tyne, UK) was used to analyze the gels and their respective activities. The Phoretix gel analysis software (Phoretix Ltd, Newcastle Upon Tyne, UK) was used to analyze the gels and their respective activities. The Phoretix gel analysis software (Phoretix Ltd, Newcastle Upon Tyne, UK) was used to analyze the gels and their respective activities. The Phoretix gel analysis software (Phoretix Ltd, Newcastle Upon Tyne, UK) was used to analyze the gels and their respective activities. 

**Identification by MALDI-TOF Mass Spectrometry**

Extracted peptide digests were dried at 30 ºC using a vacuum centrifuge. The depleted samples (100 µg for Panels A and B, and 200 µg for Panel C) were separated on 2-DE gels as described in the Methods section. Panels D, E, and F are magnifications of the Apo A1 train from gels A, B, and C respectively. With the inclusion of a protease inhibitor cocktail, many human plasma protein isoforms were revealed, which could be further analyzed by MALDI-TOF mass spectrometry. The inhibition of endogenous proteolytic activity in the intact sample via the use of a protease inhibitor cocktail provides a significant advantage in the detection of protein isoforms. The characterization of the Apo A1 isoform train was accomplished by MALDI-TOF MS evaluation of peptides derived from each of the isoforms present on 2-DE gels. Table 1 gives a comparative tryptic peptide list for each isoform. Four of the nine Apo A1 peptides identified were found to have a partner peptide with a molecular weight increase of 0.93 Da, suggesting the occurrence of specific proteolysis within the protease inhibitor cocktail (see Table 1). The spots in this train have molecular masses and pI's similar to Apolipoprotein A2.

**Conclusions**

Protease inhibitors are commonly used in protein analysis systems to profile proteins from endogenous and exogenous sources. In this study, we used a protease inhibitor cocktail to analyze the plasma protein profile of human plasma samples prepared by Western blotting (1) and MALDI-ToF MS (2). The depletion of total plasma samples (9) was achieved by IgG depletion. The depleted samples (100 µg for Panels A and B, and 200 µg for Panel C) were separated on 2-DE gels as described in the Methods section. Panels D, E, and F are magnifications of the Apo A1 train from gels A, B, and C respectively. With the inclusion of a protease inhibitor cocktail, many human plasma protein isoforms were revealed, which could be further analyzed by MALDI-TOF mass spectrometry. The inhibition of endogenous proteolytic activity in the intact sample via the use of a protease inhibitor cocktail provides a significant advantage in the detection of protein isoforms. The characterization of the Apo A1 isoform train was accomplished by MALDI-TOF MS evaluation of peptides derived from each of the isoforms present on 2-DE gels. Table 1 gives a comparative tryptic peptide list for each isoform. Four of the nine Apo A1 peptides identified were found to have a partner peptide with a molecular weight increase of 0.93 Da, suggesting the occurrence of specific proteolysis within the protease inhibitor cocktail (see Table 1). The spots in this train have molecular masses and pI's similar to Apolipoprotein A2.