Elsewhere in this issue, Jankowski et al show that mononuclear cells produce sufficient angiotensin II (Ang II) to stimulate Ang II type 1 receptors. That finding alone is noteworthy; however, my attention was riveted by the methods the authors used to measure Ang II. Ang II is generally measured by radioimmunoassay. Because of antibody specificity, the samples are first purified by high-performance liquid chromatography (HPLC). The assay is difficult, tedious, and fraught with pitfalls. The sample collections, standardization of collection conditions, and use of appropriate inhibitors are only some of the problems. Only a few investigators do this assay well. An alternative, more accurate, method would be most welcome. Were the method sufficiently established, and were the method routinely available, direct Ang II measurements could be of great clinical utility. After reading the article and meeting with the senior author, I hedge the hope that matrix-assisted laser desorption/ionization (MALDI–time of flight (TOF)) might bring this wish to reality.

Jankowski et al adapted MALDI-TOF to measure Ang II or other small peptides and proteins with interesting biological activities in a previous study. In their article, they outlined a MALDI-TOF–based strategy to screen protein fractions for defined enzymatic activities. The authors prepared porcine kidney extracts that were then immobilized by covalent coupling to activated affinity beads. The immobilized proteins were incubated with probes specific for different enzyme activities. The resultant products were analyzed by MALDI mass spectrometry. The group then verified the presence of 5’ nucleotidase, kinase, phosphatase, glutathione reductase, and renin activities in the extract. The renin activity was verified by the presence of MALDI-TOF–detected Ang I and Ang II. Imagine what Tigerstedt and Bergmann might have done with that. The findings in this proof-of-principle renal extract study were expected. However, the authors recently used the same approach to make a novel finding in the supernatant obtained from mechanically stimulated human dermal vessel endothelial cells. They identified uridine adenonsine tetraphosphate and showed that the material is a novel endothelium-derived vasoconstrictive factor.

In 2002, Koichi Tanaka, a fellow of the Shimadzu Corporation, received the Nobel Prize for Chemistry. Tanaka received the prize for the development of soft desorption ionization methods for mass spectrometric analyses. His MALDI-TOF methodology greatly facilitated the identification of biological macromolecules. However, the road to success was a long one. Twenty years earlier, Tanaka and associates developed a laser microprobe TOF mass spectrometer that laser-irradiated a sample to ionize it, put it to flight, and then measured the mass according to the TOF. However, when the laser was used on biological macromolecules, the laser decomposed them into fragments. For this reason, the laser method was thought to be impossible for analyzing macromolecules. Tanaka et al then added cobalt as an ultra fine metal powder to the matrix. Serendipitously, Tanaka also added glycerine to the cobalt. With this matrix, Tanaka was then able to identify the peaks he needed to in turn unravel the molecular structures. Tanaka’s work helped make the development of proteomics possible.

How is Ang II measured with MALDI-TOF? The method is summarized briefly in the Figure. The Ang II–containing sample, embedded in a matrix (60 μg/mL α-4-hydroxyacinamic acid in water), is crystallized. The target is mounted on an x-y-z movable stage. The target is then bombarded with a 337-nm and 3-ns pulse duration nitrogen laser. The laser beam is focused on the target. The reaction products hurtle toward the detector. Their mass is a function of the time (TOF) required for the fragments to get there. Figure 2 of the Jankowski et al article is revealing. MALDI-TOF identified a material with a molecular weight of 1046.5 (top). That value fits pretty well with the octapeptide Ang II. For the identification of component peptides (bottom), the postsource decay MALDI-mass spectrometric or LIFT-TOF-TOF MALDI techniques were used. First, all the peptides of interest (those coming from the parent compound; in this case, Ang II) were separated in the ion source of the mass spectrometer. Only the peptides of interest were introduced into the mass analyzer of the mass spectrometer. Therefore, the fragment ions detected by the mass detector were generated during the analysis procedure and could therefore be distinguished from endogenous peptide fragments that did not pass through the mass analyzer. The “breakdown” products allowed identification of Ang 1-7, Ang 3-8, or any other amino acid combination produced by the bombardment. The amount present of each fragment is a function of peak height shown on the ordinate. The mass of the fragment is given on the abscissa. Thus, endogenous Ang II fragments present in the sample were not a confounding variable.
The proof of the pudding is shown in Figure 5D of the authors’ article. Jankowski et al collaborated with Karl Hilgers, who measured their samples by radioimmunoassay. A head-to-head comparison of the two methods was then possible. What we see is a linear relationship over the range of lower-limit radioimmunoassay to high levels. Jankowski et al used an HPLC step before the samples were crystallized in matrix for MALDI-TOF. The Figure does not reveal the profoundly sensitive detection limit of MALDI-TOF, which lies in the 0.1 to 1.0 fmol/L range. The high protein content of plasma would appear to make the HPLC step seem reasonable. The authors have not tested whether or not the method has utility without this step, but studies are in progress. This work is extremely important because the HPLC step is the limiting factor for most laboratories. In any event, the HPLC step would appear to be unnecessary in studies involving interstitial or renal tubular fluid.

Clinicians could clearly use an Ang II assay, although reliance on plasma renin activity has served us fairly well.

Helmer discovered most of what we know about renin and Ang II in normal subjects, hypertensive patients, and patients with renal artery stenosis. Helmer managed this tour de force by relying solely on a bioassay. Haber et al, Boyd et al, and, soon thereafter, other groups developed radioimmunoassays for Ang I that brought plasma renin activity into routine clinical use. Direct Ang II measurements have been slow in coming. MALDI-TOF could change that situation. Once the assay is established, MALDI-TOF should allow precise Ang II measurements to be conducted in a grand scale.

MALDI-TOF relies on ion generation by directing pulsed laser light onto the matrix-embedded, crystallized sample. The ion beam is then focused and directed at the detector. The mass-to-charge ratio can be estimated from the TOF. The technique allows molecular mass determinations sufficiently sensitive to detect Dalton differences in molecular weight.

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Friedrich C. Luft

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