Spectral analysis of peptides and proteins using diode-array detection for important structural information

Biopharmaceutical

Peptides and proteins consist of a unique sequence of 20 different naturally-occuring amino acids. They differ in length, amino acid composition and secondary and tertiary structure, all of them being a unique feature and essential and characteristic for their individual physiological and enzymatical activity.

All amino acids have an absorption maximum below 220 nm, and the maximum of the peptide bond between two amino acids is below 220 nm. Among the 20 naturally-occuring amino acids only two, tyrosine and tryptophan, have a characteristic absorbance at a higher wavelength with a maximum at 280 nm. Especially tryptophan is of high interest, because it is only encoded by a single codon. Hence, whenever an unknown protein has to be characterized–typically by peptide mapping of the purified protein and internal sequence analysis–peptides containing a tryptophan residue have to be selected and analyzed by peptide sequencing. To differentiate tyrosine and tryptophan either zero order or even better, second order derivative spectra can be used. A specific oligonucleotide deriving from the peptide sequence can be synthezised afterwards and used for fishing the gene of interest.

Proteins are also often post-translationally modified after synthesis in the cell. Chromophoric groups such as linear or circular tetrapyrrols are often attached to enzymes such as hemoglobin, chlorophyll, phytochrome and others. For structural analysis peptides and proteins are routinely chemically modified, for example, by pyridylethylation of cysteine residues (VP-Cys) or by affinity labeling of specific residues. All these modifications show additional characteristic absorption spectra and maxima.

Therefore, identifying the amino acid residues is best done using diode-array detection rather than single or multiple wavelength detectors, because peptides and proteins can be detected at several wavelengths with high selectivity. Complete spectra in the range of 190–950 nm can be recorded in parallel. After detection, collected peptide and protein fractions can be analyzed further by mass spectrometry and/or peptide sequencing.

Figure 1 shows zero order and second order derivative spectra of tryptophan, tyrosine, VP-Cys with tryptophan, and VP-Cys. In the zero order spectra only VP-Cys and VP-Cys with tryptophan can be clearly distinguished. The spectra of tyrosine and tryptophan are very similar. Compared to tyrosine, the absorption maximum of tryptophan is a bit broader at 280 nm. Unambigiuous assignment of tryptophan and tyrosine, however, can be performed using the second derivative spectra. Tryptophan has a major minimum at 290 nm and tyrosine at 284 nm (figure 1, parts 2 and 4). VP-Cys does not show any significant minimum in the second order derivative spectrum (figure 1, part 8), whereas a peptide containing both tryptophan and VP-Cys shows the typical tryptophan minimum at 290 nm (figure 1, part 6).

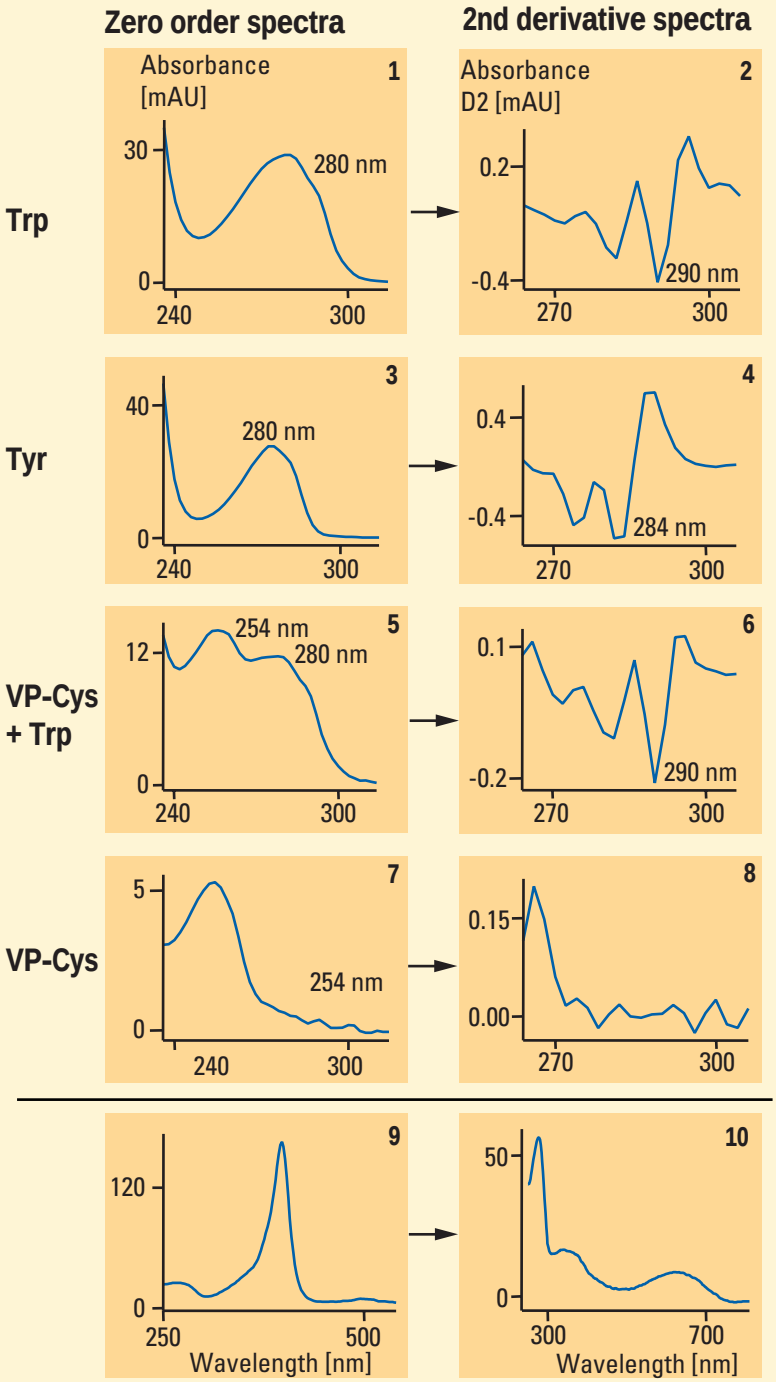
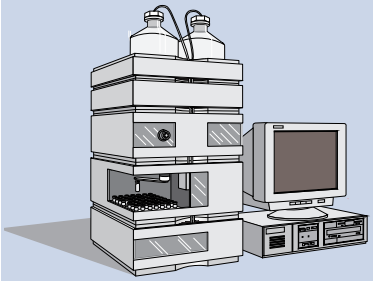


Figure 1
Peptide and protein spectra recorded with the Agilent 1100 Series diode-array detector.

A further example proving the usefulness of diode-array detection is shown in figure 1 part 9. Here the spectrum derived from hemoglobin is shown which has a characteristic major absorption maximum at 400 nm and a minor maximum at 500 nm. The spectrum shown in figure 1, part 10 derives from the absorption spectrum of phytochrome, a plant photoreceptor protein with its characteristic additional maxima at 360 nm and 640 nm.

Equipment

Agilent 1100 Series
- Binary pump (includes vacuum degasser)
- Autosampler
- Diode array detector standard or capillary flow cell
- Agilent ChemStation + 3D software



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