2 Preparation of Analytical Samples

2.1 Elemental Analysis

Over and above the usual requirements such as the elimination of chemical and mechanical impurities by recrystallization or reprecipitation [1] peptides have to be dried with exceptional care. Free peptides, often lyophilized materials, retain moisture tenaciously. In addition to water they might contain acetic acid as well. In order to obtain reproducible values the samples have to be dried in vacuo, at elevated temperature, for prolonged periods of time. The temperature of drying should be based on the melting point or decomposition point of the material. While it is desirable to choose a temperature as high as 110 °C or even 130 °C, drying should not be accompanied by melting or decomposition.

Protected peptides are usually insoluble in water. Nevertheless, they can be hygroscopic in a sense: they might absorb water from moist air. Hence, for the sake of valid analyses such samples must be dried at 110 to 130 °C and allowed to cool in a desiccator in the presence of phosphorus pentoxide. In particularly difficult cases the sample should be weighed and dried, by the microanalyst, in a “piggy”, which is closed while still hot and reweighed after cooling.

Probably because of the presence of weak basic centers, such as nitroguanidines, the imidazole of histidine or the amide groups themselves, protected peptides can contain acetic acid or trifluoroacetic acid, even after drying. This possibility should be considered at the evaluation of the value of elemental analysis. For best information all the elements present in the compound should be determined and the sum should amount to 100%.

Lengthy considerations and experience taught the authors to value the results of combustion analysis. Spectral methods should complement but not replace the determination of elemental composition.

2.2 Amino Acid Analysis

If the synthetic material is available in sufficient amounts, a 3–5 mg sample should be used and weighed with an accuracy of 0.1 mg. This allows the calculation of “recovery” from the values of amino acid analysis and thus the
determination of the peptide content of the analyzed substance. The peptide content of a sample can be far below 100% since peptides, both free and protected, can retain significant amounts of moisture and/or non-volatile inorganic impurities.

Most commonly, constant boiling hydrochloric acid is used for hydrolysis. This is prepared by diluting concentrated hydrochloric acid with an equal volume of water and distilling the mixture from an all-glass apparatus. The first part of the distillate about 10% is discarded and about 10% of the acid is left undistilled. The distillate, about 5.7 N HCl, is stored in several small glass-stoppered bottles or in sealed ampoules to prevent the absorption of ammonia from the air during frequent opening.

The sample is weighed into a soft glass ampoule with a narrow neck and dissolved in a small volume (e.g. 0.5 ml) of constantly boiling hydrochloric acid. The solution is cooled in an ice-water bath, evacuated with the help of a water aspirator and sealed under vacuum by melting the narrowed part of the tube with a small Bunsen burner. The sealed ampoule is placed into a cavity of an electrically heated metal block and kept at 110 °C for 16 hours.

Peptides containing valine and/or isoleucine residues [2] as next neighbors in their sequence require longer periods for complete hydrolysis, sometimes as long as 3–4 days. On long hydrolysis, however, significant decomposition of serine and threonine takes place. Therefore, for a precise determination of the amino acid ratios both short and long hydrolyses are necessary. From several analyses, e.g. after hydrolysis for 16, 32 and 64 hours, the true values for valine and isoleucine can be found by graphical extrapolation to infinite time while extrapolation to zero hour gives the hypothetical amount of serine and threonine in the hydrolysate in the absence of decomposition [3].

Tryptophan is destroyed in hot hydrochloric acid, particularly in the presence of air and heavy metal impurities. With highly purified hydrochloric acid, tryptophan-containing peptides can be hydrolyzed in vacuo and the tryptophan content determined [4]. Hydrolysis with 3 molar β-mercaptoethanesulfonic acid or 4 molar methanesulfonic acid causes no significant decomposition of the indole. Methionine sulfoxide is converted in part to methionine during hydrolysis with hydrochloric acid. The conversion is complete if β-mercaptoethanol (1 mg per ml) is added. The sulfoxide remains intact during hydrolysis with 3 N p-toluenesulfonic acid.

On completion of the hydrolysis the sealed ampoules are cooled to room temperature and carefully [5] opened. The hydrolysate is transferred with a pipet into a small beaker, the ampoule rinsed with distilled water and the acid is removed on a steam bath with the help of a stream of nitrogen. A small volume of distilled water is added and similarly evaporated. The residue is dissolved in a buffer of pH 2.2 and, after appropriate dilution, applied to the column of the amino acid analyzer. The applied volume depends upon the kind of instrument used. The buffer, glass vessels, pipet, etc. must be clean, especially if a sensitive method of analysis involving samples of 10 nanomoles or less is