Mass Spectrometric Determination of the Amino Acid Sequence in Peptides: Desulfurization of Sulfur-Containing Peptides

Earlier we showed that under electron impact N-acylpeptide esters containing methionine, cystine and S-carboxymethyl or S-β-aminoethyl cysteine residues largely undergo the amino acid type of fragmentation and are therefore amenable to the mass spectrometric determination of their amino acid sequence (see [1,2] and references therein). However, the mass spectra of such peptides display a large number of additional peaks, of which the most prominent are those due to elimination of the entire side chain of methionine, and to transformation of cystine or cysteine to dehydroalanine residues. The general pattern of the mass spectrum becomes more complicated the larger the molecular weight of the peptide and, especially, the larger the number of its sulfur-containing amino acid residues. Moreover, the presence of cystine or cysteine residues greatly lowers the volatility of the compounds, leading to considerable thermal destruction during the mass spectrometric determination. Finally, the presence of the sulfur-containing amino acids frequently leads to the absence of a number of sequence information peaks, which complicates still more the decoding of the mass spectrum, limiting the applicability of the method.

In order to overcome these difficulties, we attempted desulfurization of the sulfur-containing peptides by Raney nickel. Investigation of this reaction on a large number of peptides showed, however, that under the usual conditions (refluxing for several hours in an inert solvent, as recommended by LEDERER et al.[3]) a large number of side reactions take place which often bars the use of this method in combination with the mass spectrometric determination of amino acid sequence. For instance, under the above conditions (solvent-dioxane) tyrosine and tryptophane residues are often reduced to the corresponding hexa- and octahydroderivatives, whereas N-acylpeptide esters containing the octahydrotryptophane residues with a free imino group no longer undergo the amino acid type of fragmentation under mass spectrometric conditions. Furthermore, Raney nickel often strongly absorbs the peptides (particularly those containing histidine) and sometimes splits the initial peptide to compounds of lower molecular weight.

We have found that quite good results can be obtained if the desulfurization is carried out in dimethylformamide solution at 20° for 2 days in the presence of a tenfold amount by weight of the catalyst. Under such conditions methionine, cystine and cysteine (or S-substituted cysteine) residues are converted into α-aminobutyric acid and alanine residues, respectively, while tryptophane, tyrosine, histidine, pyrimidylornithine and other amino acid residues are unaffected. It is convenient to use for the desulfurization N-acylpeptide esters prepared by the method described earlier[1,4]. The N-acylpeptide esters are

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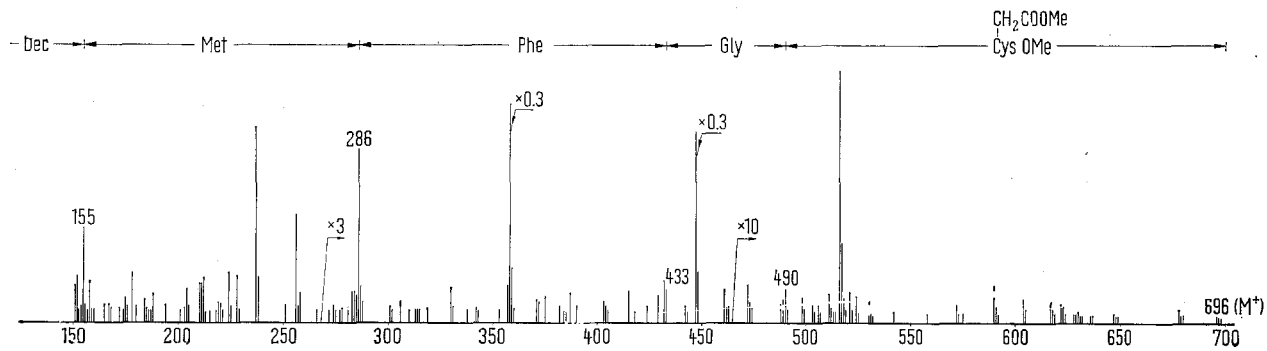

Fig. 1. Mass spectrum of Dec-Met-Phe-Gly-Cys(CH_2COOMe)-OMe (1).

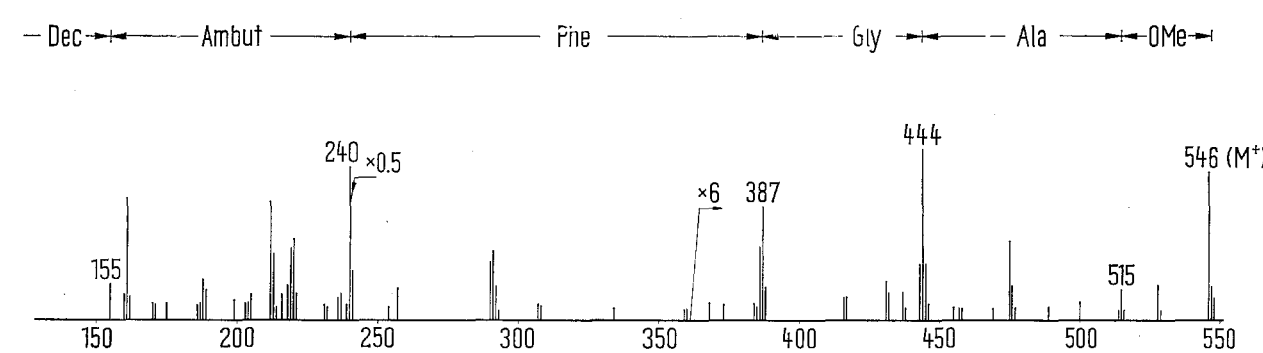

Fig. 2. Mass spectrum of Dec-AmBut-Phe-Gly-Ala-OMe (2), prepared by desulfurization of compound (1).

much less absorbed by the Raney nickel than the free peptides, and treatment of the reaction mixture is reduced to only its filtration and evaporation, the product being subjectable to mass spectrometry without purification. The α-aminobutyric acid residue behaves similarly to other aliphatic amino acids[1] under electron impact and its position in the peptide chain (and consequently that of the methionine residue) can readily be determined. If the peptide contains an alanine residue as well as cysteine (cystine) the Ni/Al alloy used for preparing the catalyst should be leached in D_2O so that the cysteine (cystine) residue is converted into deuteroalanine residue.

Figure 1 shows as example the mass spectrum of Dec-Met-Phe-Gly-Cys(CH_2COOMe)-OMe (1) which is very complicated and moreover difficult to reproduce owing to thermal decomposition of this substance during mass spectrometry. On the contrary, the mass spectrum of Dec-AmBut-Phe-Gly-Ala-OMe (2) prepared by treatment compound (1) with Raney nickel as described above, is simple and quite readily deciphered. It is also noteworthy that the temperature of mass spectrometry of this substance is 100 °C below that required for compound (1).

Hence desulfurization considerably simplifies the mass spectrometric determination of the amino acid sequence of the sulfur-containing peptides and at the same time extends the limits of this method.

Выводы. Показано, что десульфурирование эфиров серусодержащих N-ацилпептидов никелем Ренея в диметилформамиде при 20° существенно упрощает определение аминокислотной последовательности масс-спектрометрическим методом и одновременно расширяет границы этого метода.

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Electrophoretic Behavior of Sonicated Human Serum Proteins

Although gaseous cavitation has been widely used to disrupt cells and subcellular particles[1], little is known about its influence on serum or plasma proteins. Preliminary studies[2] indicate such forces render fibrinogen molecules incapable of forming a fibrin clot and solubilize lipoproteins through the uptake of additional protein[3].

Qualitative or quantitative details about changes in serum or plasma proteins mediated by gaseous cavitation are still lacking. Therefore, attempts were made to characterize the phenomenon by investigating sonic effects on the electrophoretic behavior of proteins in normal and abnormal sera as well as purified preparations of human serum albumin, γ-globulin and β-lipoprotein.

Materials and methods. Sera from normal subjects and patients with various diseases were transferred in 2.5 ml aliquots to heat-resistant glass tubes placed in an ice-water bath. A titanium probe (0.75 inches in diameter, end ratio 3.6:1) was immersed 1–2 mm below the surface of the serum and sound frequencies of approximately 20 kc/sec were generated with an MSE disintegrator[4] for 10 or 20 min. Samples remained several degrees below room temperature during sonic oscillation.

Serum before and after exposure to ultrasound were analyzed during the Micro Zone[5] electrophoretic system. Electrophoretic distributions on cellulose acetate were determined densitometrically after staining the strips with Ponceau S. Measurements of the total protein were made in terms of the biuret color reaction.

Results and discussion. A series of 12 pools, each containing 3 different sera, were prepared from 36 healthy individuals. Each pool was subjected to electrophoresis before as well as after 10 and 20 min exposure to ultrasound. The qualitative and quantitative changes induced by sonication were similar in all samples. After 10 min of oscillation, the protein content of the α- and β-globulin zones increased (Figure 1). This change, together with a reduction in albumin and γ-globulin fractions, was more conspicuous following 20 min of ultrasound.

In a typical example, protein in the albumin zone declined from an initial value of 4.6–3.5 g/100 ml after the final period of oscillation (Table). This was accompanied by a reduction in the γ-globulin fraction to about $^1/_3$ its original value. The protein displacement occurring with 20 min of oscillation caused a decline in the ratio of albumin to globulin from 2.4 to only 1.7.

Saline solutions containing purified human serum albumin[6] (6.25 g/100 ml) and γ-globulin[6] (0.7 g/100 ml) as well as a mixture of the 2 proteins at the stated concentrations were subjected to electrophoresis before and after sonication for 10 min. Such treatment of the albumin solution caused about 16% of the protein to migrate less rapidly anodally (Figure 2). When the γ-globulin solution was treated similarly, more than 30% of the protein moved into the β-globulin zone (Figure 3). Oscillation of the γ-globulin-albumin mixture caused the appearance of only a single new position zone, residing in the β-globulin position (Figure 4). About $^1/_3$ of the total protein was displaced into this area, with little or no increase in protein-staining material in the α_1-position.

Sera collected from patients with various diseases were examined electrophoretically before and after 10 min of sonic radiation. The variability of the responses obtained is illustrated by the patterns secured with specimens from 2 subjects with multiple myeloma. Sera from these patients both showed a typical peak of myeloma protein in the γ-globulin area. Following 10 min exposure to ultrasound, the pattern from 1 patient indicated a marked displacement of protein into the β- and α_2-globulin zone,

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