Dimethyl Sulfoxide and Amino Acid Analyses[1]

L. S. BATES and C. W. DEYOE[2], Department of Grain Science and Industry, Kansas Agricultural Experiment Station, Manhattan

ABSTRACT

The deleterious effects of trace amounts of dimethyl sulfoxide on peptide-bound and free amino acids during acid hydrolysis were examined. Interference was eliminated via lyophilization and use of β-mercaptoethanol.

Dimethyl sulfoxide (DMSO), an unusually versatile polar solvent, is being used increasingly in biochemical methodology. Because amino acid analyses commonly follow extraction and fractionation procedures that use DMSO, researchers must contend with DMSO removal or analysis in its presence. Elimination of deleterious effects of DMSO on free and peptide-bound amino acids during acid hydrolysis is described here.

MATERIALS AND METHODS

Two natural products, defatted, ground sorghum grain and maize pollen, were hydrolyzed *in vacuo* in a 400-fold excess of 6N HCl containing 2.5% DMSO in Pyrex tubes for 2, 8, and 24 hr. at 110° C. Untreated controls were hydrolyzed in 6N HCl. An aliquot of one sorghum sample was treated with 2.5% aqueous DMSO, lyophilized 14 hr. in a flask connected directly to an acetone-Dry Ice-cooled trap, and hydrolyzed in 6N HCl for 24 hr. An antioxidant, β-mercaptoethanol (0.08M), was added to subsequent samples, Purified tyrosine and histidine standards were mixed with the HCl-DMSO mixture and analyzed after rotary evaporation at 47° C. as well as after 2, 8, and 24 hr. of hydrolysis at 110° C.

All analyses were made on a Beckman 120-C amino acid analyzer using the standard 4-hr. method (68 ml. buffer per hr.). Total aminograms were recorded; no overlaps of short and long columns were permitted.

RESULTS AND DISCUSSION

The most striking effects of DMSO presence during acid hydrolysis were destruction of tyrosine, histidine, and arginine; increased methionine recoveries; and a cystine-to-cysteic acid conversion. Tyrosine, histidine, and arginine destruction was probably oxidative. Linked oxidation reductions could account for conversion of cystine to cysteic acid and perhaps for the effect on methionine. The linked reactions were slow; only 24 hr. hydrolysates showed measurable cysteic acid and methionine recoveries.

Arginine destruction was presumably oxidative but may involve other catalytically induced products. In some samples, apparent lysine recoveries

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[1]Contribution 791, Department of Grain Science and Industry, Kansas Agricultural Experiment Station, Manhattan 66506.

[2]Respectively: Assistant Professor and Professor.

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increased. We also occasionally observed a component trailing arginine by about 10 min. Ornithine and 3-amino-piperid-2-one (APO), occurring on alkaline hydrolysis of arginine, produce similar results. Ornithine falls indistinguishably under lysine (1) and APO elutes after arginine (2). Ornithine occurs in acid hydrolysates with elevated temperatures or long hydrolysis times, but APO, the lactam of ornithine, has not been observed under such conditions (2). Our data suggest DMSO, or a DMSO product, catalyzes arginine breakdown into ornithine and APO during acid hydrolysis. No mechanisms were proposed for these observations.

Further examination of the more serious tyrosine and histidine losses was made with purified amino acids. Free tyrosine and histidine standards having only brief contact at 47° C. with the HCl-DMSO mixture remained stable. Destruction occurred rapidly as the temperature increased to 110° C. Histidine decomposed into non-ninhydrin positive compounds which could not be traced with the amino acid analyzer. Less than 10% of the histidine peak remained after 24 hr. Tyrosine destruction was complete in 24 hr. The degradation products, labeled α through ϵ, were qualitatively traced but not identified. Product α occurs immediately before isoleucine, product β resolves between isoleucine and leucine and affects the normal resolution of the amino acids, and product γ elutes

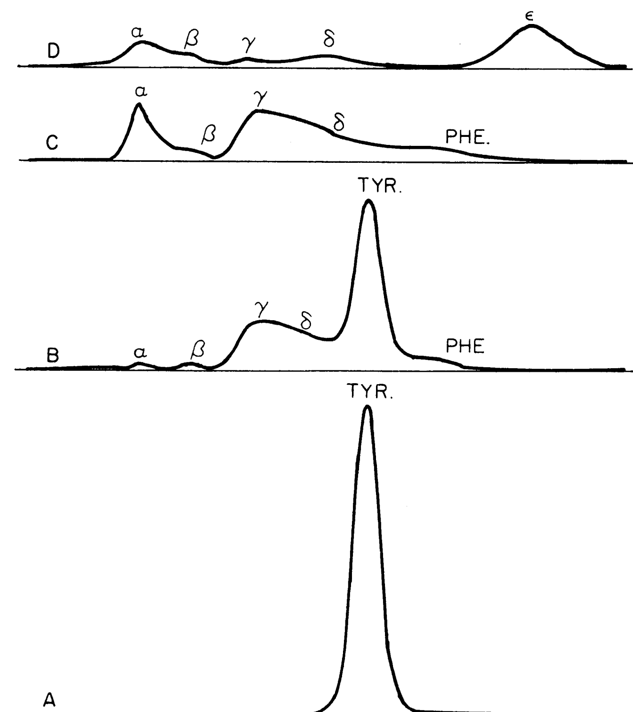

Fig. 1. Tyrosine destruction during acid hydrolysis in the presence of 2.5% DMSO. A, Standard tyrosine; 0.1M on column; B, 2-hr. hydrolysis; C, 8-hr. hydrolysis; D, 24-hr. hydrolysis.

immediately following leucine. The retention time of product δ is intermediate between component γ and tyrosine. Component ϵ separates as a distinct peak after phenylalanine. The relative elution positions are seen in Fig. 1.

DMSO, once added, is extremely difficult to separate from other reactants. Lyophilization, the best DMSO removal method for heat labile materials, was continued on one sorghum sample until it was dry and no trace of DMSO or DMS could be detected by smell. Hydrolytic destruction of certain amino acids—cystine, methionine, and tyrosine—was observed. Approximately 90% of the histidine and arginine was recovered. Dimethyl sulfoxide removal was believed incomplete. We suspect some DMSO exchanged with bound water in the sorghum flour and could not be removed despite extensive lyophilization.

Houston (3) reported the destruction of tyrosine and histidine during acid hydrolysis of proteins in polyacrylamide gel sections. The presence of 0.08M β-mercaptoethanol prevented the breakdown. With the exception of cystine, which was totally destroyed, 0.08M β-mercaptoethanol effectively prevented DMSO interference in our lyophilized samples.

Other reactions resulting from acid-catalyzed DMSO decomposition to methyl mercaptan and bis-(methylthio) methane (4) may further complicate cystine-methionine values. The presence of methyl mercaptan—an excellent antioxidant—may account for methionine recoveries when cystine was destroyed.

Trace amounts of DMSO grossly affect succeeding amino acid analyses. When dimethyl sulfoxide is necessary in operations preceding amino acid analyses, lyophilization for its removal and use of an antioxidant (β-mercaptoethanol) may minimize its interference and protect against its degradation products. The resulting aminograms should be interpreted carefully.

Literature Cited

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