[CONTRIBUTION FROM THE ABBOTT LABORATORIES]

Thiamin Stability. Effect of Amino Acids and Related Compounds and of Thiamin Concentration[1]

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The problem of thiamin stability has received attention in this Laboratory for some time.[2] That the rate of thiamin decomposition in aqueous solutions is dependent upon acidity and increases markedly with a rise in *p*H above 4 is well established.[2,3,4,5,6] There is good evidence that in pure thiamin solutions the destruction by heat is primarily a hydrolytic cleavage to give 2-methyl-5-hydroxymethyl-6-aminopyrimidine and 2-methyl-3-(β-hydroxy)-ethylthiazole.[7,8] Information is accumulating to show that the presence of other compounds in solution with thiamin may increase or decrease its rate of destruction at a given *p*H value Williams[6] pointed out that barium nitrate and sodium acetate as well as bisulfite would hasten thiamin destruction, while Beadle, *et al.*,[5] and Booth[4] have indicated that the rate of thiamin decomposition varies with different buffer salts as well as acidity. Booth[4] also showed traces of copper accelerated thiamin decomposition while several other heavy metals had no effect. Melnick, *et al.*,[9] stated that thiamin is more stable in a natural environment than in synthetic solutions and it has been reported that some proteins[10] and starch[11] markedly decrease the rate of thiamin destruction under certain conditions. It has also been reported that thiamin is quite unstable in some meat products and that the addition of certain cereals has a remarkable stabilizing effect.[12]

The purpose of this paper is to show that α- and β-amino acids have a marked stabilizing effect upon thiamin and that in pure thiamin solutions the stability depends upon thiamin concentration. The effect of amino acids is most pronounced in the presence of some salts which hasten the destruction of thiamin. The α- and β-amino acids nullify the destructive effects of such salts. Studies on the relationship of chemical structure to this stabilizing effect will be presented.

Experimental

Crystalline thiamin chloride hydrochloride and triple-distilled water were used throughout. Solutions were adjusted with dilute sodium hydroxide and hydrochloric acid to *p*H 6 ± 0.05. Solutions to be heated were sealed in 20-ml. flint glass ampules, placed in boiling water for about ten minutes, and then transferred to an oil-bath and maintained at 100 ± 1° for four hours. After heating, the ampules were cooled in ice water and kept cool until analyzed. Thiamin was determined by the thiochrome method. All figures are representative of two or more concordant experiments.

In samples containing amino acids very little *p*H change occurred during the heating period. In pure thiamin and thiamin plus sodium chloride solutions the *p*H always dropped to about 5.2 and 4.3, respectively, during the heating period.

Stabilizing Effect of α- and β-Amino Acids.—The data in Table I show that α- and β-amino acids and some of their derivatives have a marked stabilizing effect upon thiamin at *p*H 6. This effect becomes noticeable above *p*H 4.5 to 5, depending upon the concentration of thiamin and other factors, and its magnitude increases with a decrease of acidity within the range studied, namely, *p*H 4.5 to 7. As will be shown later in Table II, the stability of thiamin varies with thiamin concentration. At the concentration chosen for the data of Table I, *i. e.*, 0.1 mg. per ml., thiamin alone is much more stable than it is in the presence of sodium chloride and other salts. Consequently, at this concentration of thiamin the stabilizing effect of the amino acids is demonstrated better with thiamin plus sodium chloride than with thiamin alone.

TABLE I

RELATIONSHIP OF CHEMICAL STRUCTURE TO EFFECT ON THIAMIN STABILITY

Compound added, *M*/20	Per cent. thiamin remaining after four hours at 100°, *p*H 6: Thiamin alone, 0.1 mg./cc.	Thiamin 0.1 mg./cc. in *M*/20 NaCl
None (control)	73	57
Glycine	79	84
Glycine amide	84	84
Glycylglycine	82	85
Acetylglycine	78	34
Sarcosine	78	75
N-Dimethylglycine	..	80
Betaine	..	58
Taurine	72	78
β-Alanine	..	82
γ-Aminobutyric acid	27	22
δ-Aminovaleric acid	..	14
ε-Aminocaproic acid	..	19
Anthranilic acid	73	70
m-Aminobenzoic acid	58	59
p-Aminobenzoic acid	23	21
Choline chloride	63	57
Creatine	22	48
Acetamide	..	14
Nicotinic acid	35	23
Nicotinamide	17	13
Benzylamine	80	74
Allylamine	73	61
Diallylamine	80	77
Tributylamine	78	76
n-Butylamine	47	48
Di-*n*-butylamine	46	44

(1) Presented in part before the American Chemical Society at Pittsburgh, Pennsylvania, September 9, 1943.

(2) Frost and McIntire, THIS JOURNAL, **66**, 425 (1944).

(3) Farrer, *Australian Chem. Inst. J. & Proc.*, **8**, 113 (1941).

(4) Booth, *Biochem. J.*, **37**, 518 (1943).

(5) Beadle, Greenwood and Kraybill, *J. Biol. Chem.*, **149**, 339 (1943).

(6) Williams, *J. Am. Med. Assoc.*, **110**, 727 (1938).

(7) (a) Watanabe, *J. Pharm. Soc. Japan*, **59**, 218 (1939); (b) 500 (1939).

(8) Schöpfer and Müller, *Compt. rend. soc. biol.*, **128**, 372 (1938).

(9) Melnick, Robinson and Field, *J. Biol. Chem.*, **138**, 49 (1941).

(10) Greenwood, Beadle and Kraybill, *ibid.*, **149**, 349 (1943).

(11) Atkin, *et al.*, U. S. Patent 2,322,270 (1943).

(12) Rice, Beuk and Robinson, *Science*, **98**, 449 (1943).

TABLE II

THIAMIN STABILITY AT DIFFERENT CONCENTRATIONS, MINIMUM EFFECTIVE CONCENTRATION OF GLYCINE

Original thiamin concentration, mg. per cc.	Per cent. thiamin remaining after four hours at 100°, *pH* 6		
	No glycine	*M*/20 glycine	*M*/1000 glycine
0.001	8	70	76
.01	35	79	76
.1	73	79	79
.1 in *M*/20 NaCl	57	84	75

Several other salts including chlorides, bromides and acetates were tested and were found to increase the rate of thiamin decomposition. In general, the acetates had the greatest effect. In all cases the destructive effects of salts were nullified by glycine.

Most of the α-amino acids, including the monoaminodicarboxy and diaminomonocarboxy acids, have been tested. All except cysteine are approximately equally effective in stabilizing thiamin. The data for glycine are shown as representative. The fact that cysteine has little or no stabilizing effect upon thiamin might be owing to its sulfhydryl group since cystine is very effective even at its low level of solubility. β-Alanine was fully as effective as the α-amino acids.

Relation of Structure to Thiamin-Stabilizing Property.—The relationship of chemical structure to the thiamin-stabilizing property of amino acids was investigated with respect to (1) substitutions in the amino and carboxyl groups, (2) the relative positions of the amino and carboxyl groups, and (3) replacement of the carboxyl by other strongly negative groups. In addition, the effect of various amines and a few miscellaneous dipolar compounds was examined. The data are summarized in Table I.

The carboxyl group may be substituted to form the amide without loss of activity, *e. g.*, glycine amide. In this respect glycylglycine may be considered a substituted glycine amide. Acetylation of the amino group destroyed the thiamin-stabilizing property, but substitution of 1 or 2 methyl groups in the amino group did not. However, conversion to the quaternary ammonium base, betaine, did destroy the stabilizing quality.

The relation of effect upon thiamin stability to the relative positions of the amino and carboxyl groups is quite striking. Compounds with the amino groups on the α- or β-carbon atom had approximately the same stabilizing effect, but when the amino group was removed farther from the carboxyl, a marked destructive effect was obtained. In an amino acid having both an α- and an ε-amino group, *e. g.*, lysine, the effect of the α-amino group predominates since lysine had approximately the same effect as glycine.

The position of the amino group relative to the carboxyl is important also in the aminobenzoic acid series. Thus anthranilic acid was protective, *m*-aminobenzoic had no noticeable effect, and *p*-aminobenzoic was destructive toward thiamin.

In some compounds, *e. g.*, taurine, the carboxyl group may be replaced by the sulfonic acid group without appreciable loss of the thiamin stabilizing property.

Benzylamine and diallylamine had a definite stabilizing effect. Several saturated primary, secondary, and tertiary aliphatic amines were tested but only tri-*n*-butylamine had any stabilizing effect. All others, including *n*-butyl- and di-*n*-butylamines had a destructive effect approximately equal to that of sodium chloride.

Of practical pharmaceutical significance is the marked destructive effect of nicotinamide and nicotinic acid.

The above findings may be related to the destructive and protective effects upon thiamin in different biological materials.[9,10,12] They have no apparent relation to the enzymatic inactivation of thiamin.[13,14]

Effect of Thiamin Concentration. Minimum Effective Concentration of Glycine.—The data of Table II illustrate that in pure thiamin solutions the rate of decomposition varies with the concentration of thiamin. In the more concentrated solutions examined, thiamin was much more stable than in the very dilute solutions. The stabilizing effect of amino acids is best demonstrated at low concentrations of thiamin.

The data of Table II also show that a nearly maximum thiamin-stabilizing effect is produced by as little as *M*/1000 glycine, even at very low concentrations of thiamin or in the presence of *M*/20 sodium chloride. Concentrations of glycine lower than *M*/1000 gave irregular results regardless of thiamin concentration. No clear-cut stoichiometric relationship between thiamin concentration and minimum effective glycine concentration was found. With 0.1 mg. of thiamin per ml., an equal molar concentration of glycine, *i. e.*, *M*/3370, was effective in some experiments.

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Summary

The α- and β-amino acids decrease the rate of thiamin decomposition. This effect of amino acids is strong enough to nullify the destructive effect of certain salts upon thiamin.

Investigation of the relationship of chemical structure to the thiamin-stabilizing property revealed that γ-, δ- and ε-aliphatic amino acids and *p*-aminobenzoic have a marked destructive effect upon thiamin.

The stability of thiamin varies with the concentration. At 0.1 mg. per ml. thiamin is much more stable than at 0.001 mg. per ml.

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(13) Sealock and Goodland, THIS JOURNAL, **66**, 507 (1944).

(14) Krampitz and Woolley, *J. Biol. Chem.*, **152**, 9 (1944).