The quantitative determination of amino acid hydroxamides*

Fatty acid hydroxamides are usually determined by reaction with ferric chloride[1,2]. These methods are not as satisfactory for the determination of amino acid hydroxamides because of low color yields and large variations in the color values of different amino acids. The ferric chloride method of ISELIN, HUANG AND NIEMANN[3] was developed for the determination of amino acid hydroxamides and gives higher color yields than the method described here. However, this method is not applicable in the presence of high concentrations of hydroxylamine, because the color is bleached rapidly. The bleaching is due to the reduction of ferric ion by hydroxylamine at the higher pH of the Niemann method. Since a method was needed, where hydroxylamine would be used as a "trapping" agent[4] the modification described below was devised.

The procedure which has given best results is as follows: To 3 ml of reaction mixture at pH 7.0 (or standard solution) containing neutralized hydroxylamine (1.0 M final concentration) is added 1.4 ml of 100% trichloracetic acid, pH 0.9** and 0.6 ml of 2.0 M aqueous ferric chloride. The protein precipitate is centrifuged or filtered off and the color is read in the Beckman spectrophotometer after 5–20 minutes at 520 mμ. Blanks and standards are carried through the procedure.

The color values obtained on standards of leucine and alanine hydroxamide*** are shown in Fig. 1. Good proportionality is obtained between optical density and concentration in the range 0.2 to 1.0 μM per ml. It is not possible to obtain accurate estimations at lower concentrations than these because of the high reading of the blank (optical density is 0.08–0.1). Color values begin to fall off above 1.0 μM per ml. The values shown in the figure represent the extremes, since the determination of 4 other amino acid hydroxamides (glycine, tyrosine, tryptophan and methionine) gave similar curves which fell between the values shown. The optical densities for the 6 amino acid hydroxamides tested were 0.77 ± 0.09 at a concentration of 1 μM per ml. The concentration of hydroxylamine used does not affect the color values up to a concentration of 1.2 M. The final pH of the reaction mixture should be between 0.7–0.9. The color yield increases with higher pH, but the color fades even at pH 1.0. The pH chosen gives the highest color yield consistent with stability. Similarly, the ferric ion concentration chosen is a compromise, since higher color yields are obtained with more iron, but the blank value also increases rapidly. The high trichloracetic acid concentration gives better color yields and affords some buffering action. The method has been used with good results to follow the reaction which occurs when ATP and amino acids are incubated with a soluble enzyme from rabbit reticulocytes in the presence of hydroxylamine[5].

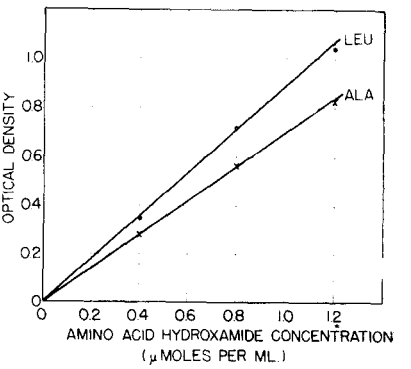

Fig. 1. Optical density readings at various concentrations of amino acid hydroxamide. The procedure is given in the text. The upper curve (●) shows values for leucine hydroxamide, the lower curve (○) for alanine hydroxamide.

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** Neutralized to this pH with sodium hydroxide. This reagent is stable for one week in the cold.

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