October 2006 Volume 3 Issue 2-3

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 3(2-3), 2006 [51-54]

A Rapid Quantitative Determination Method Of L-Ornithine

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Received: 10th July, 2006
Accepted: 26th September, 2006

Web Publication Date : 11th October, 2006

ABSTRACT

Based on Chinard's estimation, a quantitative analysis and determination of L-ornithine is established with 25mg/ml acid ninhydrin prepared with mixed acid as reagent, color development was in boiling water with 100°C for 60 minutes. A liner relationship is obtained between concentration of L-ornithine and optical density in the range 0 to 0.20 μmol per ml, nd a liner regression equation is obtained.

KEYWORDS

L-ornithine;
Ninhydrin;
Spectrophotography.

INTRODUCTION

L-Ornithine (Orn) is a non-protein amino acid, which is an important metabolite of urea cycle in mammalian, and also it is a precursor for the metabolism of L-arginine (Arg), L-proline (Pro) and polyamine. Methods for estimation and determination of Orn used such as amino acid analyzer[1,2,3], liquid chromatography[4,5,6], gas chromatography[7,8], ion-exchange chromatography[9,10], enzymatic assay[11,12,13] or spectrophotography[14,15,16,17], some defaults were subsisted such as time consuming, non-specificity or requiring purified enzyme. A method for the estimation of Orn was established with low acid ninhydrin solution by Chinard in 1952, but it was not specific because this procedure was only applicabile to pure Orn solution. Based on Chinard's method, we attempted to eliminate the interference of Pro, L-lysine (Lys) and citrulline (Cit), and established a new simple, rapid method for the quantitative determination of Orn. This procedure could be applied to estimating Orn in fermentation broth.

MATERIALS AND METHODS

L-ornithine monohydrochloric acid and Arg were purchased from Sigma Chemical Co, L-citrulline from Acros Chemical Co, other amino acids from Amersco Chmical Co, and ninhydrin from Beijing Chemical Co. Other reagents were of commercial analytical

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grade.

Preparation of acid ninhydrin reagent

25mg of ninhydrin were added per milliliter to the mixed acid solution which in milliliter contained 0.25 ml 6 mol/l phosphoric acid and 0.75 ml glacial acetic acid. After the addition of the ninhydrin, the acid mixture was heated to about 70°C to insure the dissolving of the ninhydrin. The reagent was kept in 4°C refrigerator and avoiding light.

Preparation of L-ornithine monohydrochloric acid standard solution

0.034g L-ornithine monohydrochric acid is dissolved in 100ml distilled water, and kept in 4°C refrigerator. The concentration of L-ornithine mono hydrochric acid was 2.00 μmol/ml. When used, it is diluted with distilled water appropriately.

Preparation of other amino acid standard solution

0.018g L-alanine (Ala), 0.030 g L-aspartic acid (Asp), 0.029g L-glutamic acid (Glu), 0.015g L-glycine (Gly), 0.026g L-isoleucine (Ile), 0.026g L-leucine (Leu), 0.030g L-methionine (Met), 0.033g L-phenylalanine (Phe), 0.024g L-threonine (Thr), 0.041g L-tryptophan (Trp), 0.023g L-valine (Val), 0.035g Arg, 0.023g Pro and 0.035g Cit were dissolved in 100ml distilled water respectively, and kept in 4°C.

Color development

The procedure was carried out according to Chinard (1952), except that the optical densities were determinated at 510 nm (722s spectrophotometer, Shanghai Precision & Scientific Instrument Co. Ltd. China).

RESULTS AND DISCUSSION

Specificity of acid ninhydrin to L-ornithine

The optical densities after the reaction of some amino acids with ninhydrin were shown in TABLE 1.

The other amino acid tested did not interfere except for Pro, Lys and Cit, which was in consonance with the Chinard's result.

Absorption curves of reaction products of ornithine, proline and lysine with ninhydrin

Figure 1 showed the optical densities of solutions of 0.20 μmol/ml ornithine monohydrochoric acid, Pro, Lys and Cit after reaction with low acid ninhydrin, respectively.

The curves for Orn and Pro were similar. This figure showed that the maximal absorption wavelength of the solutions of Orn and Pro after reaction with ninhydrin were 510 nm.

Effect of reaction temperature on the optical densities of solution after acid ninhydrin

The sample was separately heated in water with 60, 70, 80, 90 and 100°C for 60 minutes, then the optical densities were determined in 510 nm. Figure 2 showed the relationship between the optical densities and temperatures.

The optical densities increased when the reaction temperature increased, and OD value reached the maximum in 100°C. Therefore, under the atmospheric pressure, the most suitable reaction temperature was 100°C.

TABLE 1: The Colormetric reaction between amino acids and acidic ninhydrin reagent

AA[a]	OD_{510nm}	Color	AA	OD_{510nm}	Color	AA	OD_{510nm}	Color
Ala	0.001	-[b]	Gly	0.001	-	Phe	0.001	-
Arg	0.001	-	Ile	0.002	-	Pro	0.421	Light red
Asp	0.001	-	Leu	0.001	-	Thr	0.001	-
Cit	0.058	Pale red	Lys	0.081	yellowish	Trp	0.008	pale yellowish
Cys	0.000	-	Met	0.001	-	Tyr	0.001	-
Glu	0.001	-	Orn	0.794	crimson	Val	0.000	-

[a] The concentration of all the amino acids was 0.20 μmol/l;
[b] The color did not show.

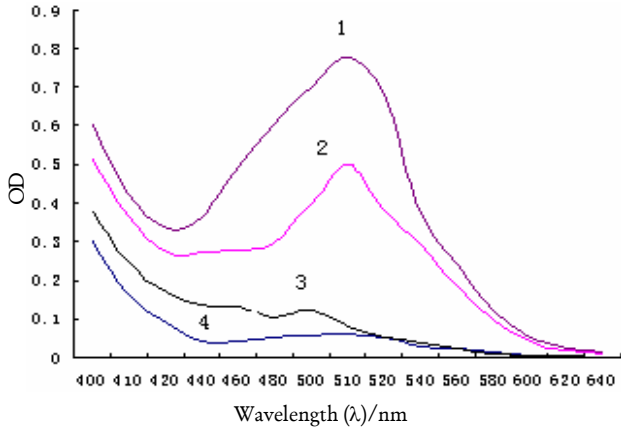

Figure 1: Absorption curves of reaction products of amino acids with ninhydrin. Curve 1, 2, 3 and 4 stand for Orn, Pro, Lys and Cit, respectively

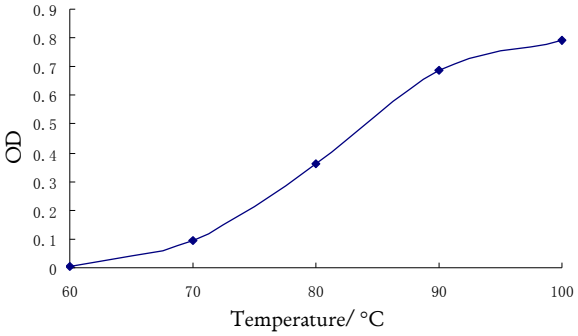

Figure 2: The optical densities of reaction solution of Orn with acid ninhydrin in different temperature. 0.20 μmol/ml Orn was used for color development

Effect of reaction time on the optical densities of the reaction solution of L-ornithine with acid ninhydrin

Based on the maxmal absorption wave-length and reaction temperature, the amino acid sample was heated in boiling water for 10, 20, 30, 40, 50 and 60 minutes, respectively. Then, the optical densities were checked. The effect of reaction time on the OD value of the reaction solution was shown in figure 3.

Figure 3 demonstrated the change of absorbance. The OD value increased rapidly at first 30 minutes and slowly at the next 30 minutes. The suitable time of color development was 60 minutes, and this could make it sure that the color developed completely.

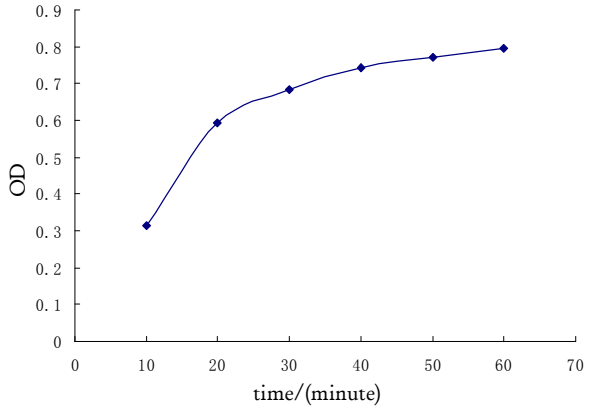

Figure 3: The relationship between OD value and the reaction time

Effect of pH value on the optical densities of the solution after reaction with acid ninhydrin

The different ratio of 6 mol/l phosphoric acid to glacial acetic acid would result in the different pH value. Therefore, 6 mol/l phosphoric acid and glacial acetic acid were mixed at ratio of 1:5, 1:3, 1:1, 3:2, and 3:1 respectively, and then used to prepare the acid ninhydrin for color development reagent. The OD value and color of the solution after Orn (0.20μmol/ml) with acid ninhydrin were shown in TABLE 2.

TABLE 2: Effect of mixing ratio of phosphoric acid-glacial acetic acid on the ninhydrin reaction

ratio	1:5	1:3	2:3	1:1	3:2	3:1
OD value	0.795	0.793	0.775	0.713	0.551	0.471
Color	+++++	+++++	++++	+++	++	+[a]

[a] + The color degree of reaction solution, reddish (+), pale red (++), red (+++), dark red (+++++), violet red (+++++).

The OD value increased and the color deepened when the mixing ratio of 6 mol/l phosphoric acid to glacial acetic acid decreased. But the OD value and color were almost identical for the ratio of 1:5 and 1:3.

Drawfting of standard curve

A strict linear relationship was obtained between Orn concentration and absorbance in the range 0 to 0.20 μmol/ml, and the solution OD could be read at least 40 minutes after reaction. Figure 4 showed

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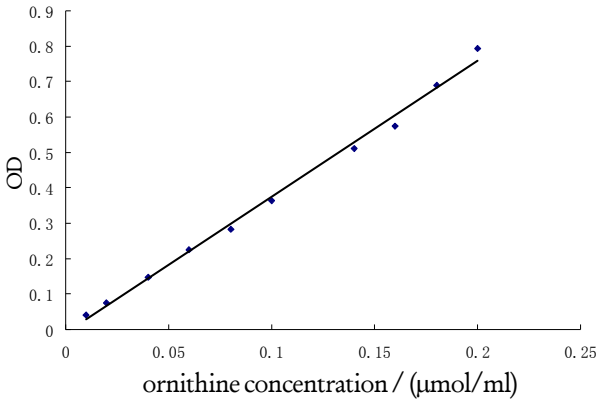

Figure 4: The linear relationship between the absorbance and Orn concentration

the relationship between the Orn concentration and optical densities.

A linear regression equation and linear correlation cofficient were obtained by using Microsoft Excel 2003 as follows:

$Y = 3.8375X - 0.0095$ (a)

$R^2 = 0.9973$ (b)

among Y: the optical density (510nm) of solution after reaction with acid ninhydrin; X: the concenstration of L-ornithine (μmol/ml); R^2: the linear correlation cofficient.

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