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MICROCALORIMETRIC CONTROL OF MICROBIOLOGICAL PROCESSES. II. ANALYTICAL DETECTION OF THE TOTAL CONCENTRATION OF AMINO ACIDS

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ABSTRACT

Microcalorimetric methods have been established for continuous analytical control of the total concentration of amino acids in solutions in biotechnological processes. The methods are based on the reactions of the complex formation of copper(II) ions with amino acids; in experiments, use was made of a flow–mix microcalorimeter. The techniques were applied to measure the total concentration of amino acids in yeast extract, peptone, HCl hydrolysates of casein and enzymatic lysates of protein–vitamin concentrate. Other typical components of biotechnological solutions (carbohydrates, ammonium ions, urea, acetate and phosphate) have virtually no influence on the results of the determinations.

INTRODUCTION

Along with analytical control of the carbon sources glucose, sucrose and lactose (see paper I of this series, ref. 1) during the growing of recombinant microorganisms, it is extremely important to control the concentration of substances comprising the nitrogen source. Preparations containing amino acids are often used as nitrogen sources [2].

The literature refers to the following microcalorimetric methods for detection of amino acids: (1) oxidation of amino acids with sodium hypobromite in 0.05–0.5 *N* NaOH solution [3], and (2) titration of amino acids with detection of the endpoint by the indicator reaction of acetaldehyde condensation with acetone [4]. Both methods are insufficiently selective. For example, under the above conditions sodium hypobromite oxidizes not only amino acids but also ammonium, urea and some other substances [3,5,6]. As to the second method, it is even less selective [4,7–12].

EXPERIMENTAL

In order to determine the total concentration of amino acids during microbiological processes, we employed the reaction of complex formation

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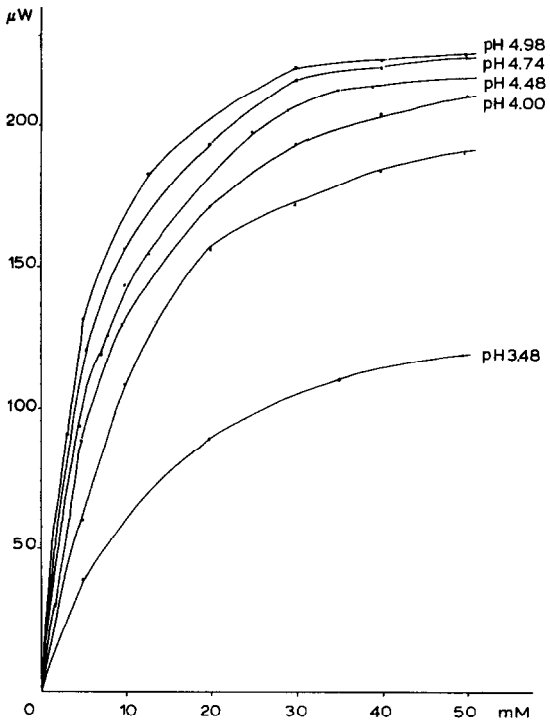

Fig. 1. Heat effect (μW) of interaction of alanine (0.005 M) with copper sulphate (0.004–0.050 M) at pH 3.5–5.0.

of amino acids with Cu^{2+} in acetate buffer. Experimental microcalorimetric detection was carried out as described in paper I of the series [1].

We varied the following parameters: pH of acetate buffer, concentration of copper ions and the most common components of biotechnological processes (ammonium ions, urea, phosphate, glucose, lactose, sucrose and some organic acids).

Figure 1 presents the values for heat effects of Cu^{2+} interaction with alanine (concentration 0.005 M) at pH 3.5–5.0. It should be explained that alanine was used for selection of the optimal conditions for microcalorimetric analysis of amino acids because the content of this amino acid in bacterial hydrolysates and some artificial mixtures is the highest as compared to other amino acids [13]. For instance, the alanine content in yeast extract A (produced by the Olaina plant of chemical reagents, U.S.S.R.) is 10 times that of other amino acids.

At low copper concentration (up to 0.03 M), a sharp increase in the heat effect with the rise in Cu^{2+} concentration is observed (Fig. 1). The concentration increase is accompanied with relative retardation of the increase in the heat effect. Thus, with the enhancement of Cu^{2+} concentration, the precision of analytical detection also increases, since the same error in

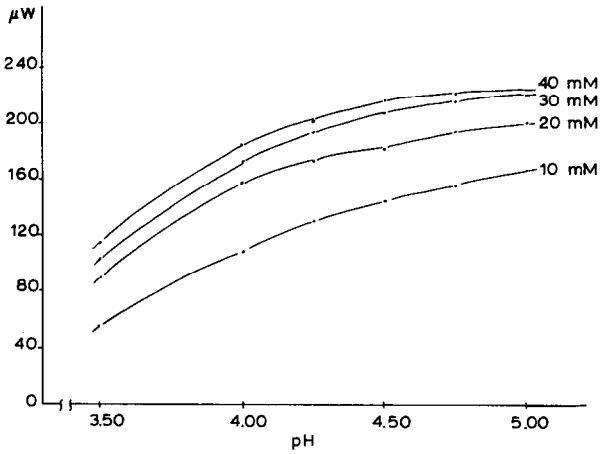

Fig. 2. Heat effect (μW) of interaction of alanine (0.005 M) with copper sulphate (0.01, 0.02, 0.03 and 0.04 M) vs. pH.

concentration upon preparing the solutions influences the results less at higher concentrations (> 0.03 M).

Interestingly, with a pH increase from 3.5–4.2, a sharp enhancement of the heat effect of the reaction takes place in the interaction of Cu^{2+} with 0.005 M alanine. Further pH increase (4.4–4.9) does not affect the heat effect. In addition, pH 4.4 is preferable since variation in the Cu^{2+} concentration (0.03–0.05 M) at this pH does not change the heat effect (Fig. 2). Figure 2 shows the dependence of the heat effect of the studied reaction of Cu^{2+} ions (concentration 0.01, 0.02, 0.03 or 0.04 M) with 0.005 *M* alanine at pH 3.5–5. Because of a sharp decrease in the buffer capacity with increasing pH (pH > 4.8), acetate buffer of pH > 4.8 is not suitable for analytical purposes.

Much attention has been paid to the study of effects of dilution of the reaction components. Figure 3 presents the dependence of heat effects of dilution of $CuSO_4$ solutions (0.005–0.05 M) at pH 3.5–5. Within the whole pH range we observed an increase in the absolute value of the heat effect with the growth in Cu^{2+} concentration. Figure 4 shows the dependence of the heat effects of dilution of Cu^{2+} solutions (0.01, 0.02, 0.03 and 0.04 M) on pH. Since our aim is to minimize the value of the heat effect of dilution (Figs. 3 and 4), the Cu^{2+} concentration should not exceed 0.035 M. In our opinion, a pH of 4.5 is optimal.

In order to show the applicability of our technique in determining the concentrations of other amino acids, we studied also leucine, lysine, arginine, valine, glycine and glutamine. According to the data presented in Fig. 5 the character of interaction of Cu^{2+} with alanine, leucine and lysine is fairly similar.

Figures 6a and 6b demonstrate calibration curves for detecting different amino acids [Fig. 6a: valine, glutamine, arginine hydrochloride; Fig. 6b:

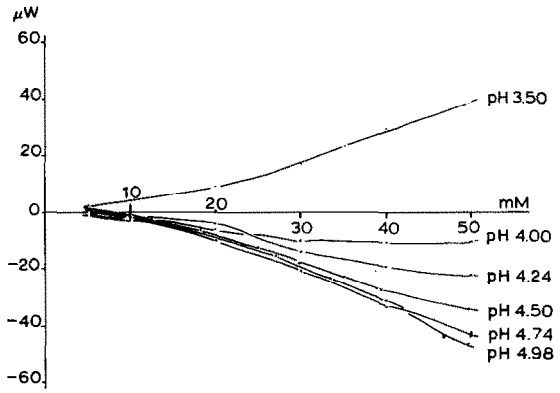

Fig. 3. Heat effects (μW) of dilution of copper sulphate (0.005–0.05 M) at pH 3.5, 4.0, 4.24, 4.5, 4.74 and 4.98.

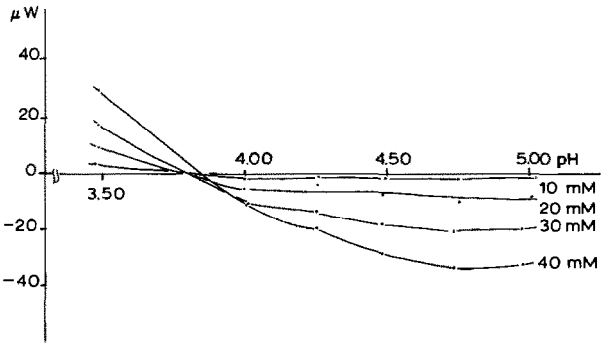

Fig. 4. Heat effects (μW) of dilution of copper sulphate (0.01, 0.02, 0.03 and 0.04 M) vs. pH.

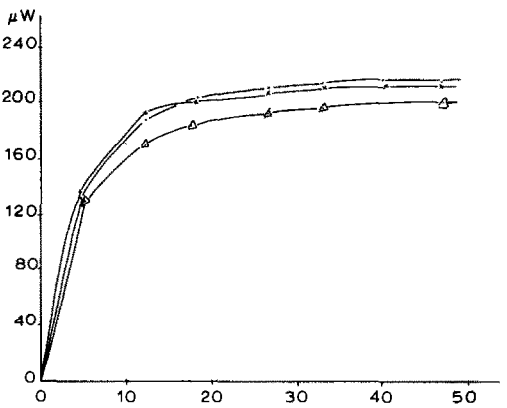

Fig. 5. Heat effects (μW) of interaction of 0.005 M alanine (●), 0.005 M leucine (×) and 0.005 M lysine (△) as a function of copper sulphate concentration (0.004–0.050 M) in acetate buffer (0.1 M, pH 4.98). The equation of the analytical reaction at high copper concentrations (0.03–0.05 M) is:

$$[Cu(CH_3COO^-)]^+ + R\text{–}\underset{\displaystyle NH_3^+}{\underset{|}{CH}}\text{–}COO^- \rightleftharpoons [Cu(R\text{–}\underset{\displaystyle NH_2}{\underset{|}{CH}}\text{–}COO^-)]^+ + H^+ + CH_3COO^-.$$

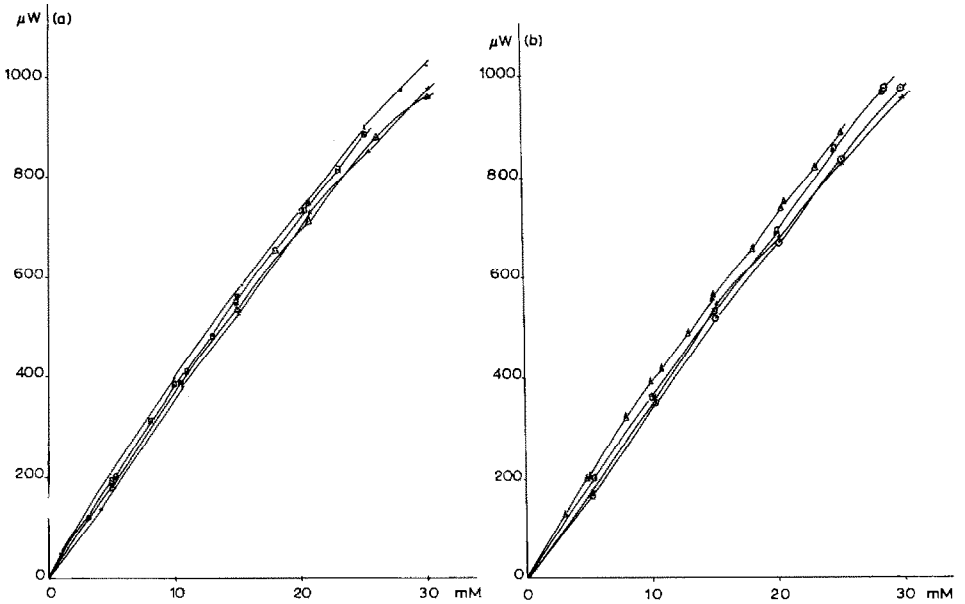

Fig. 6. a, Calibration curve for detection of concentrations of alanine (⊡), valine (●) glutamine (×) and arginine (△) relative to heat effect of complex formation of copper sulphate (0.035 M) with the amino acids in acetate buffer (0.1 M, pH 4.5). At low concentrations of amino acids, the complexes $[Cu(R-\underset{\displaystyle NH_2}{\underset{|}{CH}}-COO)^-]^+$ dominate in solution, at high amino acid concentrations, $[Cu(R-\underset{\displaystyle NH_2}{\underset{|}{CH}}-COO^-)_2]^0$ appears in solution. Since the enthalpy values for addition of amino acids to Cu^{2+} upon the addition of both the first and second amino acid molecule are close [13], the curve remains linear at high amino acid concentrations as well. b, Calibration curve for detection of leucine (⊙), lysine (⊡), alanine (△) and glycine (×) relative to heat effect of complex formation of copper sulphate (0.035 M) with the amino acids in acetate buffer (0.1 M, pH 4.5).

leucine, lysine, glycine) via heat effects of their interaction reactions with Cu^{2+} (0.035 M) in acetate buffer (0.1 M; pH 4.5). The calibration curves are very similar , and thus the technique we have elaborated characterizes the total concentration of amino acids.

When studying the influence of the components of biotechnological processes we found that urea, ammonium ions and organic phosphates (e.g. AMP), as well as conventional carbon sources (glucose, sucrose, lactose), are practically without influence on the results of analysis at the concentrations comparable with those of amino acids. As to orthophosphoric acid, the situation is more complicated. The problem was specially studied by us. As Fig. 7 demonstrates, the heat effect of the Cu^{2+} interaction reaction with orthophosphoric acid depends on the pH of the buffer. The concentration of orthophosphoric acid is ca. 0.003 M, which corresponds to the most probable concentration of this component in biotechnological processes. Figure 7 shows that at pH < 4.7 the heat effect of interaction of orthophosphoric acid with Cu^{2+} is near zero. A pH decrease prevents precipitation.

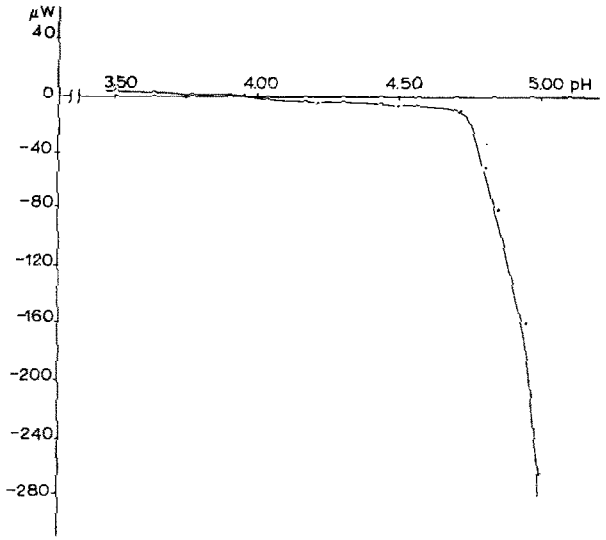

Fig. 7. Heat effects (μW) of interaction of copper sulphate (0.035 M) with KH_2PO_4 (0.03 M) vs. pH.

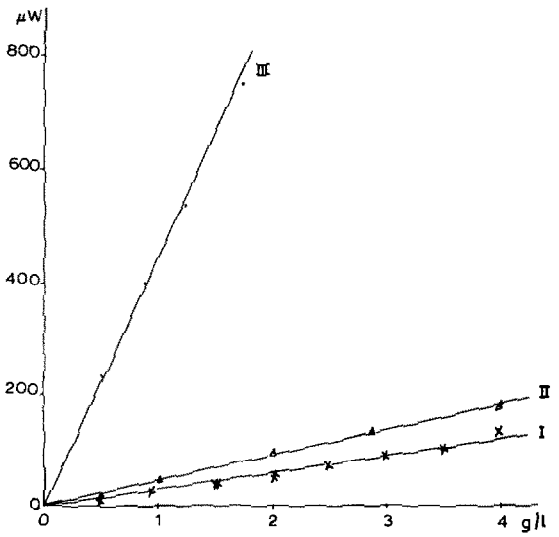

Fig. 8. Heat effects (μW) of interaction of copper sulphate (0.035 M) with: I, yeast extract A (U.S.S.R.); II, enzymatic hydrolysate of protein–vitamin concentrate (U.S.S.R.); III, alanine: The dependence is used as the calibration curve corresponding to 100% content of amino acid in the sample. The dependence is expressed by the analytical equation $W_{Ala}(\mu W) = \gamma C_{Ala}(g\ l^{-1})$, $\gamma = 420$ (l g^{-1} μW). Coefficient γ (assumed to be 100%) is applied to calculate the total content of amino acid: in yeast extract A, 7%, $\alpha = 30$ (l g^{-1} μW); in hydrolysate, 11%, $\beta = 45$ (l g^{-1} μW).

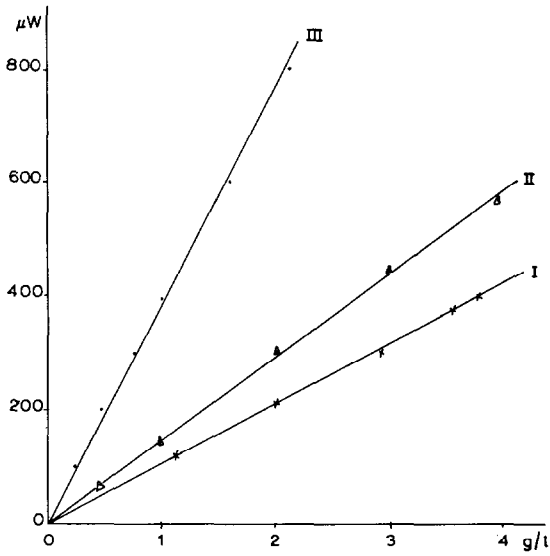

Fig. 9. Heat effect (μW) of interaction of copper sulphate (0.035 M) with: I, peptone (Czechoslovakia); II, hydrochloric acid hydrolysate of casein (U.S.S.R.); III, leucine: The dependence is used as the calibration curve corresponding to 100% content of amino acid in the sample. The dependence is expressed by the analytical equation $W_{Leu}(\mu W) = \gamma C_{Leu}$ (g l^{-1}), $\gamma = 375$ (l g^{-1} μW). Coefficient γ is assumed to be 100% and is applied to calculate the total content of amino acid: up to 28%, $\alpha = 105$ (l g^{-1} μW) in peptone; 39%, $\beta = 145$ (l g^{-1} μW) in hydrochloric acid hydrolysate of casein.

We determined the total concentration of amino acids in yeast extract A (U.S.S.R.) (Fig. 8), enzymatic hydrolysate of a protein–vitamin concentrate (U.S.S.R.) (Fig. 8), peptone (Czechoslovakia) (Fig. 9) and hydrolysate of casein (U.S.S.R.) (Fig. 9).

Figure 8 presents the values of the heat effect of Cu^{2+} interaction with yeast extract A (curve I) and with pure alanine (curve III), which is the predominant amino acid in the sample of yeast extract. Figure 8 also shows the results of microcalorimetric measurements on a hydrolysate of the protein–vitamin concentrate (curve II), which is an analogue of yeast extract A in many biotechnological processes. By comparing the slope coefficients for curves I, II, III, we found that the total content of amino acids in extract A is 7% (by wt., here and below) and that in an enzymatic hydrolysate of the protein–vitamin concentrate is 11%.

Figure 9 shows the values of heat effects of interaction of copper(II) ions with peptone (curve I) and a pure amino acid—leucine (curve III)—which is a dominating amino acid in this sample. Here also are the results of microcalorimetric detection of the hydrochloric acid hydrolysate of casein (curve II), which can serve as a peptone analogue in biotechnological processes. From the slopes of curves I, II and III we found that the total content of the amino acid in the peptone sample is 28%, and that in the hydrolysate is 39%.

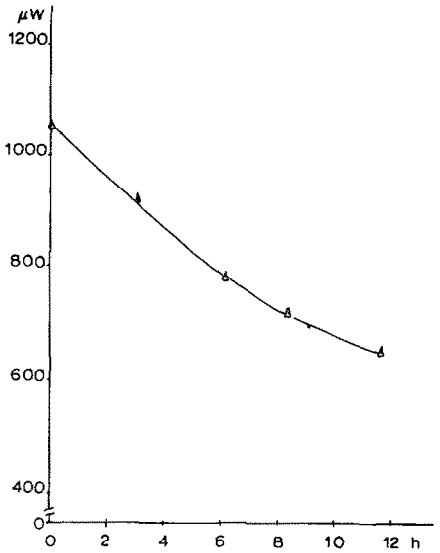

Fig. 10. Detection of the total concentration of amino acids in a typical experiment on cultivation of recombinant *E. coli*.

The described microcalorimetric procedures were applied not only to the solutions of pure amino acids (Figs. 6a and 6b) and to preparations used as the source of amino acids in biotechnology (Figs. 8 and 9) but also to culture fluids. Determination of the total concentration of amino acids in a typical experiment on the cultivation of recombinant *E. coli* (Fig. 10) shows a gradual decrease in the concentration of amino acids during the growth of microorganisms.

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