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Note

MICROCALORIMETRIC DETECTION OF L-ASPARAGINE AND L-GLUTAMINE IN SOLUTION USING L-ASPARAGINASE FROM *E. COLI*

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Application of a flow microcalorimeter considerably decreases the analysis time as compared to a batch microcalorimeter, but, however, provides no reliable detection of the substrate concentration if the enzymatic reaction proceeds incompletely. The present paper takes account of all the effects which appear with asparagine and glutamine determination in both the flow-type and batch-type microcalorimeters.

RESULTS AND DISCUSSION

We have previously developed the method for L-asparaginase activity determination using a flow-type calorimeter, LKB-2107-121 [1]. Because the reaction mixture contains a large excess of asparagine, the observed heat release is proportional to the enzyme activity in the studied solution. With a large excess of enzyme, the heat release is proportional to the asparagine concentration. This dependence affords the assay of the exact asparagine concentration in the medium, if the enthalpy of the asparagine hydrolysis is known [2,3].

The technique has an accuracy of 1–2% (analysis time 15 min) and a detection limit $\sim 5 \times 10^{-6}$ Ml^{-1} in the case of the asparagine determination. There were difficulties for the glutamine detection in solution. Firstly, the activity of *E. coli* L-asparaginase relative to glutamine is several times lower than compared to asparagine [4]. Therefore, the concentration range investigated for glutamine (0.25–4 mM, Table 1) is smaller than for asparagine (0.25–25 mM). In some experiments, the heat release was only ~ 6 μW (Table 1). For accurate measurements of these small heat effects, a more sensitive instrument was used (Bioactivity Monitor LKB-2277 [5] equipped with a flow-mix cell (LKB-2277-204), instead of a microcalorimeter LKB-2107-121).

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TABLE 1

Heat release for enzymatic glutamine hydrolysis of glutamine enzyme *E. coli* L-asparaginase, 400 IU ml^{-1}, Bioactivity monitor equipped with flow-mix cell LKB-2277-204, substrate and enzyme flow rate 2.63×10^{-3} ml s^{-1}

Calibration 30 μW (arbitrary units)	Experiment		L-glutamine concentration (mol ml^{-1} $\times 10^{-7}$)	L-glutamine flow rate mol s^{-1} $\times 10^{-9}$)	Heat released (per mol of glutamine) (kJ mol^{-1})
	(in arbitrary units)	(in μW)			
30.7	94.0	91.9	40.0	10.52	8.74
30.7	51.6	50.4	20.0	5.26	9.58
30.7	28.0	27.4	10.0	2.63	10.42
30.7	23.6	23.1	8.33	2.19	10.55
31.0	10.3	10.0	3.76	0.989	10.11
31.0	6.3	6.1	2.28	0.600	10.17
30.7	6.3	6.2	2.28	0.600	10.33
30.7	10.9	10.7	3.76	0.989	10.82
30.7	23.0	22.5	8.33	2.19	10.27

Secondly, the data in Table 1 indicate the proportional relation between the heat release and the glutamine concentration in solution. In the range 0.2–1 mM, the enthalpy of glutamine is -10.4 kJ mol^{-1}. Thus the heat effect is 2.5 times lower than the average enthalpy of the amide bond hydrolysis [3,6].

If the flow rate v of glutamine and L-asparaginase through the flow-mix cell decreases, the enthalpy per mole of glutamine increases as follows: -10.4 ($v = 7.51 \times 10^{-4}$ ml s^{-1}); -15.9 ($v = 1.50 \times 10^{-3}$ ml s^{-1}); -20.5 ($v = 7.51 \times 10^{-4}$ ml s^{-1}); -24.6 ($v = 3.76 \times 10^{-4}$ ml s^{-1}). Consequently, despite the proportional dependence between the heat release and the glutamine content in solution (Table 1), the hydrolysis proceeds incompletely. Complete hydrolysis occurs only when the flow rate decreases or (the same thing) when the reaction mixture remains inside the cell for longer.

The data listed in the table refer to the incomplete hydrolysis and so can change depending on a variety of factors: the presence of enzyme activators and inhibitors in solution, the pH, the composition of the medium, the ionic strength, etc.

Therefore, we conclude that L-glutamine can be more reliably assessed by static "batch" microcalorimetry rather than by the dynamic flow technique: in the former, the reaction is complete.

For analytical purposes, we have designed a simple "batch" cell operating jointly with a Bioactivity Monitor LKB-2277 (see our other paper in this issue). Using this cell, the glutamine concentrations in the solutions analysed (sample volume 0.3–0.4 ml) were determined with an accuracy of ~ 3%.

In principle, virtually complete hydrolysis of glutamine can also be achieved with a flow-mix microcalorimetric cell, e.g. the LKB-2277-204. In this case, at the first stage of analysis the substrate and enzyme solutions are fed into the cell at the rate of $2.5–3.5 \times 10^{-3}$ ml s^{-1}. The high rate diminishes the time the solutions need to reach the reaction zone of the cell. After solution-mixing in the cell begins, the feeding rate decreases to 3.76×10^{-4} ml s^{-1}. As shown earlier, practically, all the glutamine undergoes enzymatic hydrolysis. The average enthalpy of the amide bond hydrolysis should be used for estimation of the glutamine concentration in solution, in case of both the flow-mix and the "batch" cells [3,6].

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