Available online at www.sciencedirect.com
SCIENCE DIRECT®
Thermochimica Acta 416 (2004) 71–74
thermochimica acta
www.elsevier.com/locate/tca

A study of promotive and fungistatic actions of steroidal saponin by microcalorimetric method

Honglin Zhang*, Xiufang Yu, Xiaoxia Li, Xiaoru Pan

Department of Chemistry, Qufu Normal University, Qufu 273165, PR China

Received 19 October 2002; received in revised form 30 November 2002; accepted 30 December 2002

Available online 27 February 2004

Abstract

In this paper, the steroidal saponin was isolated from natural Chinese drugs of yam and allum macrostemon bunge. The promotive and fungistatic actions of steroidal saponin was studied. The power–time curves of growth of *Escherichia coli* at different concentrations of steroidal saponin were determined by using a 2277 Thermal Activity Monitor. The rate constant of promotive and fungistatic actions was calculated. The relationship between growth rate constant with concentration was established. The optimum concentration of promotive action and the minimum concentration of fungistatic action were determined. Results have proved that the steroidal saponin of yam and allum macrostemon bunge has promotive action (in low concentration) and fungistatic action (in high concentration).

Keywords: *E. coli*; Fungistatic action; Promotive action; Steroidal saponin; Growth rate constant; Microcalorimetry

1. Introduction

Saponin is a very important bioactive substance; its sapogenin is the chief material in synthetic steroidal contraceptive drug and hormonal drug. With the continuous development of isolated technique and structural research, saponin chemistry has developed fast, and new bioactive substances can be found continuously. The biological activity and medical value of saponin, such as its anticancer and fungistatic property, have been attracting attention. In recent years, many researchers [1–4] in chemistry and pharmacy have discussed the isolation, extraction and chemical character of saponin's constituent, and have studied the biochemical and pharmacological actions of saponin. Saponin is divided into two groups: steroidal saponin and triterpenoidal saponin. But research on the promotive and fungistatic activities is still negligible.

In previous papers [5,6], we have reported the fungistatic action of a synthetic medicine and the promotive action of a ginseng by the microcalorimetric method.

In this paper, we isolated steroidal saponin from natural Chinese drugs of yam and allum macrostemon bunge, and studied the promotive and fungistatic actions of steroidal saponin. The power–time curves for growth of *Escherichia coli* were determined at different concentrations (c) of steroidal saponin. The rate constants (μ) of promotive and fungistatic actions are calculated. The μ versus c relationship is established. The optimum concentration of promotive action and minimum concentration of fungistatic action were determined. Research on the promotive action (in low concentration) and fungistatic action (in high concentration) of steroidal saponin has not been reported.

2. Experimental

2.1. Instrument

A 2277 Thermal Activity Monitor (Thermometric AB, Sweden) was used to determine the power–time curves of *E. coli* growth. With this instrument, reactions can be carried out in the temperature range 10–90 °C. It was maintained at a temperature within $\pm 2 \times 10^{-4}$ °C.

The detection limit was 0.15 μW and the baseline stability (over a period of 24 h) was 0.20 μW. The performance of this instrument and the details of its construction have been described previously [7].

2.2. Materials

Bacteria *E. coli* was employed. A beef extract soluble medium (pH 7.2–7.4) was used, containing NaCl (1 g), peptone (2 g), beef extract (1 g) and different concentrations of

* Corresponding author.

0040-6031/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.tca.2003.11.033

steroidal saponin of yam or allum macrostemon bunge in each 200 ml. The isolated method of steroidal saponin was given in literature [8,9].

2.3. Method

The complete cleaning and sterilization procedure for the flow tubing were as follows: sterilized distilled water, 0.10 mol l^{-1} HCl, 0.10 mol l^{-1} NaOH and 75% ethanol solution were pumped through the system for 30 min at a flow rate of 30 ml h^{-1}. Finally sterilized distilled water was pumped through the system for 30 min at a flow rate of 10 ml h^{-1} and the baseline was determined. After a stable baseline was obtained, the bacterial sample, medium and steroidal saponin solution of different volume were pumped into the flow cell system, and the monitor began to record the power–time curves of continuous growth for *E. coli*. When the recording pen returned to the baseline, the process of bacterial growth was completed.

3. Theoretical model

Bacterial metabolic process was determined under isothermal and isochoric conditions; supply of nutrient matter and oxygen was limited, the growth process of *E. coli* is inhibited and the exponential model [10] could not be used in this process.

In the growth phase, theoretical model is in accordance with the following law:

$$\frac{dN_t}{dt} = \mu N_t \pm \beta N_t^2 \quad (1)$$

where μ is the growth rate constant, β the promotive or fungistatic rate constant and N_t the bacterial number at time t; plus (+) represents the promotive process and minus (−) represents the fungistatic process.

If the power produced by every bacterium is P_0, then

$$P_t = P_0 N_t \quad (2)$$

and accordingly

$$\frac{dP_t}{dt} = \mu P_t \pm \left(\frac{\beta}{P_0}\right) P_t^2 \quad (3)$$

The integral of Eq. (3) is given by

$$P_t^{-1} = \left(P_0^{-1} \mp \frac{\beta}{\mu P_0}\right) e^{-\mu t} \pm \frac{\beta}{\mu P_0} \quad (4)$$

Using the experimental data of P_t and t obtained from the bacterial growth curves, according to Eq. (4), the growth rate constants (μ) were calculated.

4. Results and discussion

4.1. In the presence of steroidal saponin of yam

These power–time curves were determined at 37 °C and different concentrations of steroidal saponin of yam for *E. coli*. The parts of curves were shown in Fig. 1.

From the data of P_t and t, according to Eq. (4), the growth rate constants (μ) were calculated. The data were shown in Table 1.

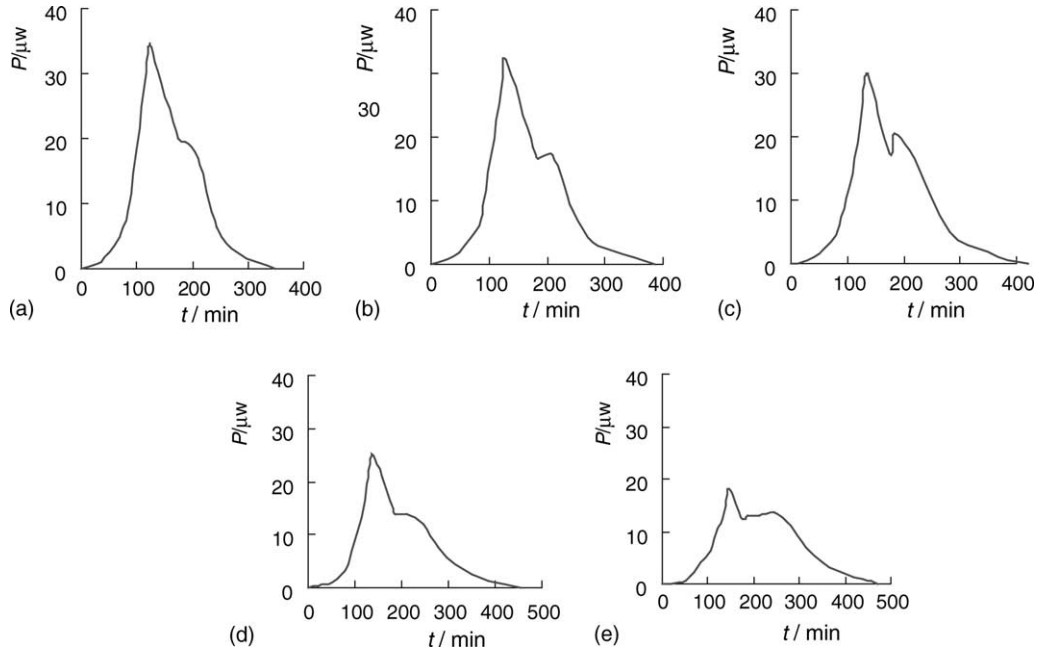

Fig. 1. Power–time curves at 37 °C and different concentrations of steroidal saponin of yam for *E. coli*: (a) $c = 1.17 \times 10^{-5}$ g ml^{-1}; (b) $c = 5.81 \times 10^{-5}$ g ml^{-1}; (c) $c = 0$; (d) $c = 2.25 \times 10^{-4}$ g ml^{-1}; (e) $c = 3.20 \times 10^{-4}$ g ml^{-1}.

Table 1
Growth rate constants (μ) at different concentrations of steroidal saponin of yam

c ($\times 10^5$ g ml^{-1})	μ (min^{-1})	r
0	0.0377	0.9990
1.17	0.0433	0.9989
2.34	0.0432	0.9920
3.51	0.0425	0.9900
5.81	0.0407	0.9890
11.25	0.0382	0.9950
16.88	0.0360	0.9932
22.50	0.0341	0.9975
28.13	0.0323	0.9980
32.00	0.0310	0.9970

The μ versus c curve obtained by data in Table 1 was shown in Fig. 2. When the range of concentration of steroidal saponin of yam is 0 to 11.25×10^{-5} g ml^{-1}, the μ versus c was established:

$$\mu = -9 \times 10^{-5}c^4 + 1.1 \times 10^{-3}c^3 - 5.2 \times 10^{-3}c^2 + 9.5 \times 10^{-3}c + 0.377$$

When μ has a maximum value, $C_a = 1.70 \times 10^{-5}$ g ml^{-1}, where C_a is the optimum concentration of promotive action.

When $c > 11.25 \times 10^{-5}$ g ml^{-1}, the μ versus c relationship can be expressed as

$$\mu = 0.04201 - 34.838c, \qquad r = -0.9989$$

When $\mu = 0$, $C_c = 1.21 \times 10^{-3}$ g ml^{-1}, where C_c is the minimum concentration of fungistatic action. $C_b = 11.25 \times 10^{-5}$ g ml^{-1} is the concentration at the interface point.

In the concentration range 0–C_b, steroidal saponin of yam is promotive action for *E. coli*, and when $c > C_b$, steroidal saponin of yam is fungistatic action for *E. coli*.

From these data, the optimum concentration (C_a) of promotive action and minimum concentration (C_c) of fungistatic action were determined.

4.2. In the presence of steroidal saponin of allum macrostemon bunge

For the case of the experiment at 37 °C and different concentrations of steroidal saponin of allum macrostemon

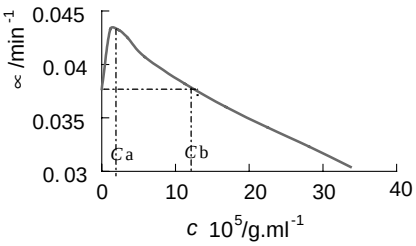

Fig. 2. The μ vs. c curve at 37 °C and different concentrations of steroidal saponin of yam.

Table 2
Growth rate constants (μ) at different concentrations of steroidal saponin of allum macrostemon bunge

c ($\times 10^3$ g ml^{-1})	μ (min^{-1})	r
0	0.0377	0.9990
0.89	0.0442	0.9995
1.78	0.0473	0.9991
2.37	0.0483	0.9993
3.54	0.0460	0.9989
4.74	0.0427	0.9989
8.77	0.0367	0.9996
10.62	0.0348	0.9985
14.22	0.0314	0.9990
17.23	0.0287	0.9992
21.24	0.0256	0.9994

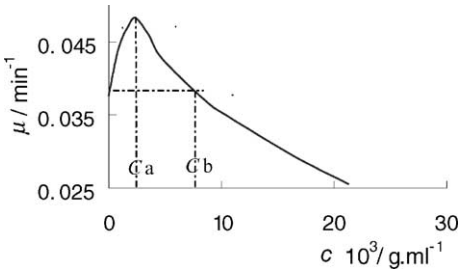

Fig. 3. The μ vs. c curve at 37 °C and different concentrations of steroidal saponin of allum macrostemon bunge.

bunge, the results in Table 2 and Fig. 3 were obtained. Similarly, from these data we obtained the following relationship.

When the values of c are 0 and 8.00×10^{-3} g ml^{-1}

$$\mu = 2 \times 10^{-4}c^3 - 2.8 \times 10^{-3}c^2 + 9.9 \times 10^{-3}c + 0.0377$$

When μ has a maximum value, $C_a = 2.80 \times 10^{-3}$ g ml^{-1}.

When $c > 8.00 \times 10^{-3}$ g ml^{-1}

$$\mu = 0.04431 - 0.89283c, \qquad r = -0.9987$$

When $\mu = 0$, $C_c = 0.0496$ g ml^{-1}. C_b, the concentration at the interface point, is 8.00×10^{-3} g ml^{-1}.

Comparing the results of promotive and fungistatic actions of yam and allum macrostemon bunge, we found that in the presence of low concentration of steroidal saponin, it is promotive action, and in the presence of high concentration of steroidal saponin, it is fungistatic action, and at the same concentrations of steroidal saponin of yam and allum macroscemon bunge, it is clear that promotive and fungistatic actions are yam > allum macrostemom bunge.

5. Conclusions

Steroidal saponin was isolated from natural Chinese drug. The power–time curves of *E. coli* at different

concentrations of steroidal saponin of yam and allum macrostemon bunge were determined using a Thermal Activity Monitor. On the basis of the experimental results and theoretical model, the growth rate constant, optimum concentration and minimum concentration were calculated. The results have proved that the steroidal saponin of yam and allum macrostemon bunge has promotive action in low concentration and fungistatic action in high concentration.

Acknowledgements

Supported by the Natural Science Foundation of Shandong Province.

References

[1] L. Xu, Acta Pharmacol. Sin. 4 (1982) 314.
[2] J. Hu, China J. Chin. Mater. Med. 11 (1995) 607.
[3] Y. Jang, N. Wang, X. Yao, Acta Pharmacol. Sin. 5 (1998) 355.
[4] Y. Hong, G. Zhang, L. Sun, Acta Pharmacol. Sin. 7 (1999) 518.
[5] H. Zhang, Z. Nan, H. Sun, Y. Liu, L. Xu, Q. Shan, X. Sun, Thermochim. Acta 223 (1993) 23.
[6] X. Yu, H. Zhang, Z. Li, H. Hang, M. Lu, J. Yuan, J. Therm. Anal. 50 (1997) 499.
[7] J. Suurkuusk, I. Wadsö, Chem. Scripta 20 (1982) 155.
[8] Q. Lin (Ed.), Traditional Chinese Medical Component and Chemistry, Scientific Publishers Limited, Peking, 1977, p. 398.
[9] C. Xiao, Traditional Chinese Medical Chemistry, Shanghai Scientific and Technical Publishers, Shanghai, 1997, p. 163.
[10] P. Gao, Y. Qu, X. Qin, B. Jang, W. Yan, Microorganism Growth and Fermentation Technology, Shandong University Press, Jinan, 1990, p. 20.