B

(2011 / 3 / 14 2010 / 10 / 19)

B

(Sephadex G-100)

(24,000 ± 500 Da)

B

(100 mmol/L)

N-Benzoyl-DL-

(1 mmol/L)

(40°C)

(6.0) pH

-

(BANA) Arginin-β-Naphthylamide

.

(346.20 U/L)

(0.489 mmol/L)

-

B

-

EDTA

.

.(0.01 mmol/L)

HgCl₂

.

BANA

B

:

Isolation and Studying Cathepsin B Enzyme from Normal Human Serum

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ABSTRACT

The work included the isolation of cathepsin B enzyme from normal human serum using different biochemical techniques which included: ammonium sulfate precipitation, dialysis and gel filtration chromatography on sephadex G-100. Furthermore, the comparative molecular weight of cathepsin B enzyme which is partially isolated from serum was found to be $(24,000 \pm 500 \text{ Da})$ by gel filtration chromatography.

The results also showed that the optimum conditions of cathepsin B enzyme activity were obtained using sodium phosphate (100 mmol/L) as a buffer at pH (6.0), at a temperature (40°C) and (1 mmol/L) of BANA as a substrate. Using Linweaver Burk plot, it was found that $V_{\max}$ and K_m have the values of (346.20 U/L) and (0.489 mmol/L) respectively.

The results also showed that the activity of cathepsin B enzyme was increased using thiol compound. Cysteine showed a greater activation than β -mercaptoethanol. The activation of cathepsin B enzyme was greater in the presence of EDTA in the reaction mixture.

Finally, the effect of iodoacetate, calcium chloride, mercury chloride, zinc sulfate and potassium cyanide on the activity of cathepsin B was studied. These compounds showed strong inhibition on the activity of cathepsin B. The inhibition of cathepsin B by mercury chloride was irreversible type of inhibition at a concentration (0.01 mmol/L).

Keywords: Cathepsin B, isolation, human serum, BANA, inhibition.

Lysosomes

1920

(Turk *et al.*, 2002) Mammals

.(Chwieralski *et al.*, 2006) Lysosome

X W U T S O N L K H F C B

Cysteine Cathepsin

Serine

E D

Aspartic Cathepsin

Monomeres

.G A

Cathepsin

.....

B

(35,000 Da) (20,000 Da)

.(Greenspan *et al.*, 2001)

(EC 3. 4. 22. 1) B

Thiol-Dependent

Papain Family

B

(Bansal and Kidwal, 1979)

B

.(Sarba, 2008)

.(Musil *et al.*, 1991) 1991

B

Glycoprotein

B

(Asn 110/113)

264

(Taniguchi *et al.*, 1985 ; Ritonja *et al.*, 1985)

(27,500 Da) (29,000 Da)

(5,000 Da) Light Chain

(Val 44/48) (Asn 43/47)

(24,000 Da) Heavy Chain

(Cys 25/29)

.(Kuhelj *et al.*, 1995)(Higashi *et al.*,1986)(Okitani *et al.*,1988)(Moin *et al.*, 1992)

B

.(Foghsgaard *et al.*, 2001)

B

(Sarba, 2008)

(Myers *et al.*, 2007)

B

33

(25 °C) Plain Tube
(900 xg) (15 min) Centrifuge
()
B

(Stern *et al.*, 2004)

B

- (Barrett, 1977)

BANA N-Benzoyl-DL-Arginin- β -Naphthylamide

- .

.(Olonen, 2004)

- B

(A*) -

(Olonen, 2004) -

- (1) -

. { A* = A_{Test} - A_{blank} } (A*)

(1 μ mol) (U)

(1 ml)

.(

.(μ mol of β -Naphthylamine/min/mg of protein)

(Lowry *et al.*, 1951) -

.(Schacterle and Pollack, 1973)

. - :1

B

B

.(Matsumoto *et al.*, 1983) (2)

.(30 min) (4000 xg)

(pH = 5 ,10

- (0.1%) (100 mmol/L) mmol/L)

B

. B :2

Dialysis

(4 C°) (Plummer, 1978) (0.1 M)

(12 h)

(48 h)

(-20 C°)

Gel

(100.0 × 2.0 cm) filtration chromatography

(Sephadex G-100)

(314 ml) (150,000 Da)

(Elution Solution) (95.0 cm)

(B)

(Andrews, 1965)

(Bovine Serum Albumin, α-Amylase, Egg Albumin, Pepsin, Papain)

: B

(1) B

()

() Sephadex G-100

(3) (

(1) (24.6) B

B

(247.0 ml) (24,000 ± 500 Da)

(4) (Azaryan *et al.*, 1985) (Barrett, 1973)

(Sephadex G-100)

: 3

.(95 cm)

Sephadex G-100

(100 $\times$ 2.0 cm)

(4 min)

(75 ml/h)

..... B

B

.B ()

) EDTA

B (5 4

.(2)

. B :2

(mmol/L)	BANA (mmol/L)	(C°)	(Sod. Pi buffer) EDTA +	(min)	(mg/ml)
4.0	1.0	40.0	(pH=6.0 , 100 mmol/L) + 1.5 (mmol/L)	15.0	2.5

- (Lineweaver-Burk plot) -

(346.20 U/L) (V_{max}) (0.489 mmol/L) Michaelis Constant (K_m)

.(5) B

B - :5

B

B (MCE) - EDTA

EDTA B

(3)

B -

.(Towatari *et al.*, 1979) (4)

.....

B

B

EDTA

:3

* %	EDTA (mmol/L)	(mmol/L)
100.0	0.0	0.0
110.0	0.2	0.0
110.0	0.5	0.0
110.0	0.78	0.0
277.1	0.0	0.1
367.5	0.0	0.2
662.9	0.0	0.3
675.8	0.0	0.4
677.2	0.0	0.5
3440.1	0.78	0.1
466.7	0.78	0.2
843.6	0.78	0.3
847.3	0.78	0.4
846.3	0.78	0.5

*(%100) EDTA : % *

(EDTA) - :4

B

* % -	* %	(mmol/L)
100.0	100.0	0.0
187.4	423.4	0.2
301.9	770.2	0.4
441.7	772.8	2.0
689.3	771.3	4.0
690.1	772.6	6.0

*(%100) EDTA : % *

B

HgCl₂

-

: 6

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