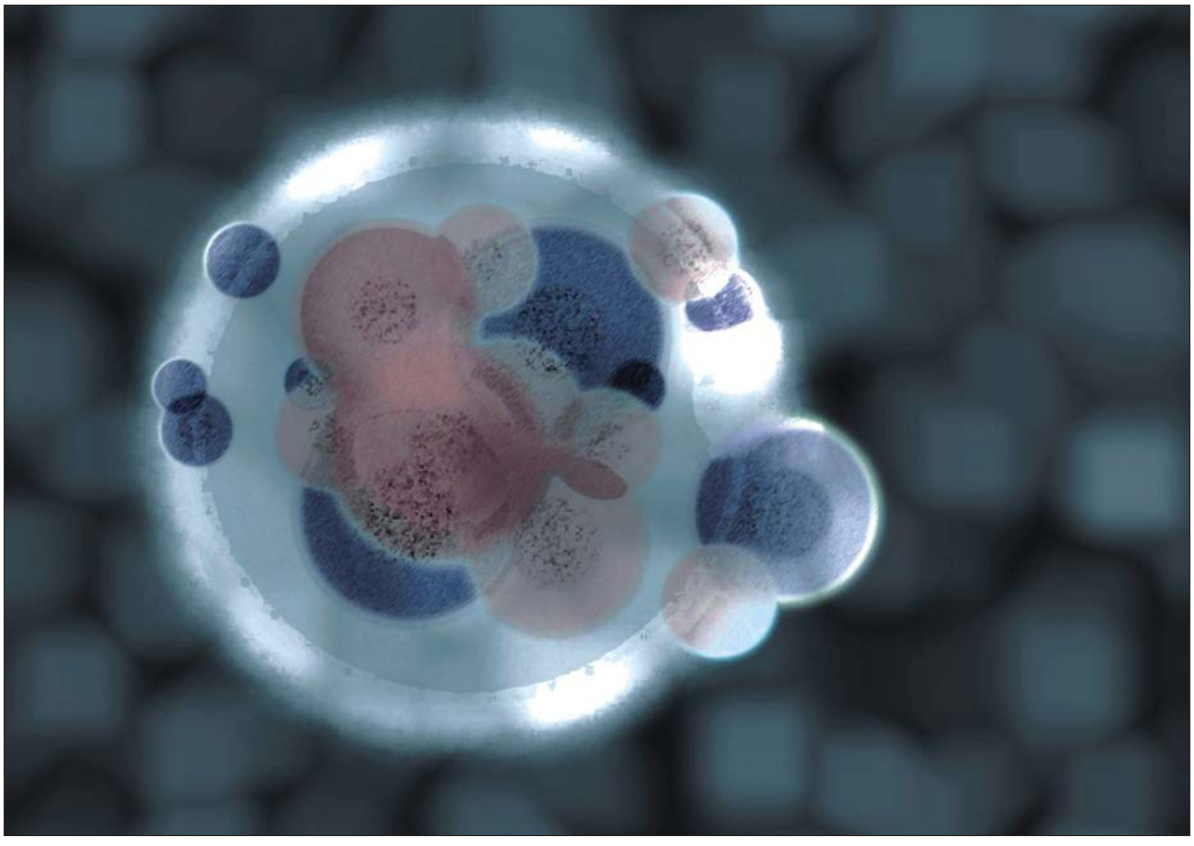


Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 6 (5): 905-910, 2006
ISSN 1727-3048

Extent and Mode of Degradation of RNA Monomers by Extracts of *Penicillium politans* NRC 510

Thanaa H. Ali
Department of Microbial Chemistry, National Research Center, Dokki, Cairo, Egypt

Abstract: Extracts of *Penicillium politans* NRC 510 catalyzed dephosphorylation of CMP, UMP, GMP and AMP optimally at pH 6 and 40°C. They also catalyzed deamination of cytidine and adenosine out of the tested ribonucleotides, ribonucleosides and bases. Neither cleavage of the N-glycosidic likages of these nucleotides nor those of the corresponding nucleosides could be effected by the extracts. Phosphate liberation from the four RNA monomers was effected by phosphate-non repressible acid phosphatase. The affinity of the phosphatase for the different ribonucleotides was in the order of magnitude CMP > UMP > GMP > AMP. Freezing and thawing of the extracts had no effect on the activity of the phosphatase or on that of the aminohydrolase. However, heating the extracts at 60°C for 30 min, in absence of the substrate, inactivated the phosphatase and increased the activity of the deaminase about 50%. No evidence for the involvement of specific nucleotidases in ribonucleotides dephosphorylation was recorded. Separation of the phosphohydrolase from the aminohydrolase was achieved using Sephadex G-100 column chromatography.

Key words: *Penicillium politans* phosphatase, ribonucleotides degradation, RNA monomers degradation

INTRODUCTION

As far as the author is aware, the mode and the extent of RNA monomers degradation by extracts of organisms belonging to the filamentous fungi has been reported for extracts of very few organisms non of which is a *Penicillium politans*. These organisms are *Penicillium chrysogenum* (Elzainy *et al*., 1979), *Aspergillus niger* strain (Elzainy *et al*., 1989) and *Aspergillus niger* $NRRL_3$ (Ali and Elzainy, 2000). The aim of the present study was to investigate the mode and the extent of degradation of these important biomolecules by extracts of a non previously studied organism which is *Penicillium politans*. Such study may add to the area of comparative biochemistry concerning this class of microorganisms.

MATERIALS AND METHODS

Materials: AMP, GMP, CMP, UMP, adenosine, guanosine, cytidine, uridine, adenine, guamine, cytosine and uracil are products of Sigma, phenyl disodium orthophosphate (phph) was a product of BDH Chemicals Ltd and Sephadex G-100 is from Pharmacia Fine Chemicals.

The organism: The *P. politans* NRC-510 strain was from the culture collection of the Department of Microbial Chemistry, National Research Center, Cairo, Egypt.

Medium: *P. politans* was grown and kept on slants of solid modified Czapek Dox's medium containing (g L^{-1} tap water): glucose, 30; $NaNO_3$, 2.0; KH_2PO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5; KCl, 0.5 and agar 20.

Methods

Preparation of *P. politans* extracts: The 4 days old mats, grown on liquid modified Czapek-Dox's medium at 28°C were harvested by filtration, washed thoroughly with distilled water, then blotted dry with absorbent paper. The mats were then ground with cold washed sand in a chilled mortar and extracted with cold distilled water. The slurry so obtained was centrifuged at 5500 rpm for 10 min and the supernatant was used as the crude enzyme preparation.

Colorimetric determination: Pi was measured as described by Ames (1966). Reducing compounds were determined (as ribose) by the method described by Ashwell (1957). Protein of the extracts was estimated by the method of Sutherland *et al.* (1949) with bovine serum albumin as standard and that of the eluted fractions was estimated by UV absorption according to the method of Layne (1957). Ammonia was determined by its nesslerization according to Vogel (1961).

Chromatographic analysis: Separation and identification of the ribonucleotides, the ribonucleosides and the bases were carried out by ascending paper chromatography

technique using 3 MM (46×57 cm) filter paper and two solvent systems. The solvent systems used for identification of the intermediates and products formed during AMP, GMP, CMP and UMP dephosphorylation by the phosphatase of the extracts were solvent I which consisted of n-butanol: acetone: acetic acid (glacial): NH_3 (5%): H_2O, 45: 15:10: 10: 20 and solvent II which consisted of n-butanol: acetic acid (glacial): water: 70: 20: 10 (Smith and Seakins, 1976).

Separation of the acid phosphatase from the aminohydrolase using Sephadex G-100 column chromatography: The crude extracts were heated at 50°C for 10 min and then immediately cooled in ice, after which they were centrifuged at 12000 rpm for 10 min at -20°C. The Sephadex G-100 column (1.5×40 cm) was equilibrated with 0.02 M Tris-acetate buffer at pH 6 and then loaded with 10 mL of the supernatant of the pre-heated extracts. Fractions of 5 mL each were collected at room temperature (25°C) at a flow rate of about 26 mL h^{-1}. At the end of the fractionation, the activity of each fraction was tested with phenyl phosphate and cytidine as substrates.

The assay reaction mixture of the eluted fractions contained (in 1 mL vol.): 0.4 mL of each fraction, 5 µmole substrate and 80 µmole Tris-acetate buffer at pH 6. The time of the reaction was 30 min and the temperature was 40°C.

Specific activity was expressed as µmoles phosphate or ammonia released from 5 µmole substrate per mg protein per 30 min at 40°C.

Appropriate control reaction mixtures where the enzyme source or the substrate was omitted were used as blanks through out the study.

Each experiment cited in this study was repeated at least five times and all the results were reproducible.

RESULTS AND DISCUSSION

Hydrolysis of RNA monomers by extracts of *P. politans*: Figure 1 shows that when the extracts were incubated with each of AMP, GMP, CMP and UMP as substrates at different pH values (from pH 3 to 9) phosphate was liberated in all the reaction mixtures and its liberation was optimum at pH 6. The same figure also shows that the pH-activity profiles obtained with the four substrates were similar to the analogous profile recorded with the synthetic substrate phenyl phosphate. Figure 2 presents phosphate release at the optimum pH as function of the temperature of the reaction mixtures. From these data collectively it was suggested that phosphate release was effected by an acid phosphatase. It can also be suggested

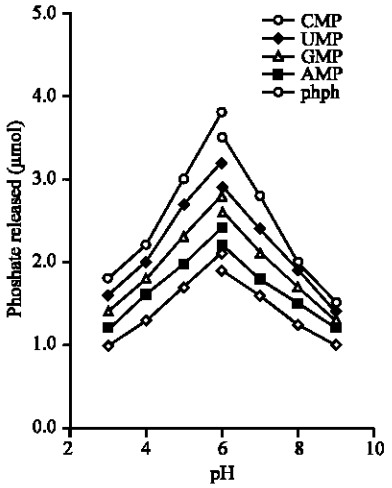


Fig. 1: pH-dependence of the phosphatase activity. Reaction mixture contained: substrate, 5 mM; extract protein 1.89 mg; buffer, 80 mM (citrate pH 3-6 and Tris-HCl pH 6-9); total vol., 1 mL; temp., 40°C; incubation period, 30 min

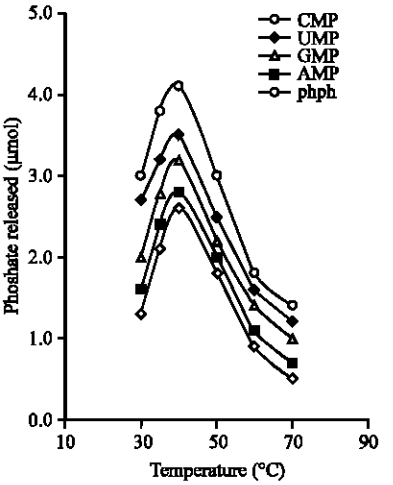


Fig. 2: Temperature-activity relationships of the acid phosphatase. Reaction mixture contained: substrate, 5 mM; buffer, 80 mM Tris-acetate pH 6; extract protein 2.12 mg; total vol., 1 mL; temp., as indicated and incubation period, 30 min

from the results of the Fig. 1 and 2 that the activities of the dephosphorylating enzyme(s) with the five substrates were in the order of the magnitude CMP> UMP> GMP> AMP and phph.

Extents of degradation of RNA monomer by *P. politans* extracts: Results of Table 1 show the extents of degradation of the four ribonucleotides under the optimum conditions of pH and temperature. It appears

Table 1: Extents of ribonucleotides degradation by extracts of *P. politans*

	Products (μmole)		
Substrate	Pi	Ribose or ribose-5-phosphate	NH_3
CMP	5.00	0.0	4.20
UMP	4.90	0.0	0.00
GMP	4.88	0.0	0.00
AMP	5.00	0.0	4.10

Reaction mixture contained : substrate, 5.0 μmole; protein, 3.2 mg; buffer, Tris-acetate pH 6, 80 μmole; vol., 1 mL; time, 180 min; temp., 40°C

that all the Pi that resides in each of the four ribonucleotides was released by the end of the incubation period (Table 1). This amount of Pi seems to be liberated directly from the nucleotide molecule and not from an intermediate in the way of its degradation such as ribose-5-phosphate or a deaminated nucleotide. This suggestion was based on the absence of reducing compounds and also the absence of equimolar amounts of NH_3 from all the reaction mixtures. However, an amount of NH_3 represented about 80% of that which originally resides in the amount of the substrate used was detected (at the end of the incubation period) in the reaction mixture that contained CMP and AMP as a substrate at pH 6. From this last result, it was suggested that NH_3 was liberated from the cytidine and adenosine formed from CMP and AMP, respectively, by the action of the phosphatase of the extracts. Chromatographic analysis of all the reaction mixtures at the end of the incubation period did not reveal appearance of any base.

Sequence of Pi and NH_3 release during CMP and AMP degradation: In order to determine the sequence of release of Pi and NH_3 during CMP and AMP degradation, the two reaction mixtures containing CMP and the other containing AMP as substrate were colorimetrically and chromatographically analyzed at different time intervals over a period of 3 h. Figure (3, 4) and Table (2, 3) show that Pi liberation preceded NH_3 release during CMP and AMP degradation. This suggests that CMP and AMP were degraded to uridine and inosine, respectively, via the intermediate formation of cytidine and adenosine. Data presented in Table 2 are in accordance with this suggestion as they show chromatographic appearance of cytidine prior to uridine during the degradation process and that UMP was not detected throw out the incubation period, also data in Table 3 show chromatographic appearance of adenosine prior to inosine and IMP was not detected throw out the incubation period.

Further confirmation for the existence of this pathway can be observed from results cited in Table 4 which demonstrate that incubating CMP or AMP with extracts containing heat inactivated acid phosphatase (this was achieved by heating the extracts at 60°C for 10 min) or by

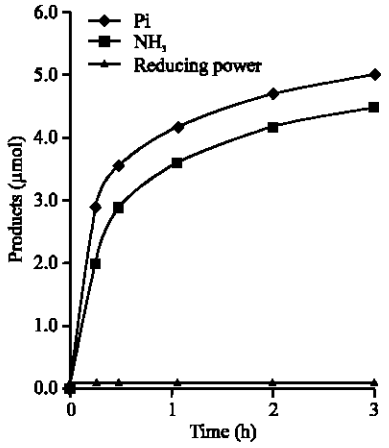


Fig. 3: Extents of Pi and NH_3 liberation from CMP at pH 6. Reaction mixture contained: substrate, 5.0 mM; buffer, 80 mM Tris-acetate pH 6; extract protein 2.42 mg; total vol., 1 mL; temp. 40°C; incubation period, as indicated

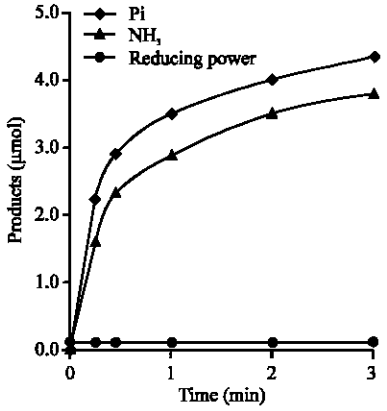


Fig. 4: Extents of Pi and NH_3 liberation from AMP at pH 6. Reaction mixture contained: substrate, 5.0 mM; buffer, 80 mM Tris-acetate pH 6; extract protein 2.42 mg; total vol., 1 mL; temp. 40°C; incubation period, as indicated

addition of sodium azide (which causes complete inhibition to the acid phosphatase) to the reaction mixtures did not lead to release of either Pi or NH_3 in the reaction mixtures. However, when the same extracts were incubated with cytidine or adenosine as substrate NH_3 was released from them. Results of the same table also indicate that the aminohydrolase activity was increased by heating the extracts to 60°C for 10 min. In this connection, freezing and thawing of the extracts was found to have no effect on either the phosphatase or the deaminase activities.

From these data ribonucleotides degradation in this organism are suggested to proceed as shown next:

Table 2: Chromatographic identification of the intermediates and products formed during CMP degradation

Compounds identified in the reaction mixture	R_f values of the compounds identified							
	Zero time		30 min		60 min		180 min	
	SI	SII	SI	SII	SI	SII	SI	SII
CMP	0.26	0.10	0.26	0.1	-	-	-	-
Cytidine	-	-	0.52	0.32	0.52	0.32	-	-
Uridine	-	-	0.57	0.4	0.57	0.4	0.57	0.4
UMP	-	-	-	-	-	-	-	-

Reaction mixture contained: substrate, 5.0 μmole; protein, 3.2 mg; buffer, Tris-acetate pH 6, 80 μmole; vol., 1 mL; time, as indicated; temp., 40°C

Table 3: Chromatographic identifications of the intermediates and products formed during AMP degradation

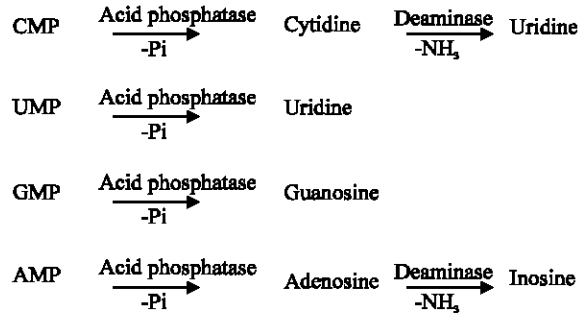
Compounds identified in the reaction mixture	R_f values of the compounds identified							
	Zero time		30 min		60 min		180 min	
	SI	SII	SI	SII	SI	SII	SI	SII
AMP	0.30	0.075	0.30	0.075	-	-	-	-
Adenosine	-	-	0.56	0.39	0.56	0.39	-	-
Inosine	-	-	0.48	0.3	0.48	0.30	0.48	0.30
IMP	-	-	-	-	-	-	-	-

Reaction mixture contained: substrate, 5.0 μmole; protein, 3.2 mg; buffer, Tris-acetate pH 6, 80 μmole; vol., 1 mL; time, as indicated; temp., 40°C

Table 4: Effect of exposing the extracts to 60°C on the phosphatase and the aminohydrolase

Substrate	Products (μmol)			
	Non heated extract		Heated extract	
	Pi	NH_3	Pi	NH_3
CMP	3.60	3.10	-	0.0
Cytidine	-	3.30	-	5.0
AMP	3.06	2.45	-	0.0
Adenosine	-	2.55	-	4.1

Reaction mixture contained: substrate, 5.0 μmole; protein, 3.2 mg; buffer, Tris-acetate pH 6, 80 μmole; vol., 1 mL; time, 30 min; temp., 40°C
*Extracts were heated at 60°C for 10 min



Absence of evidence for involvement of specific nucleotidases in Pi liberation from the four ribonucleotides: Figure 1 and 2 show that changes, in the pH and in the temperature of the reaction mixtures, revealed changes in the dephosphorylating activities with the four ribonuclotides similar to the changes recorded with phenyl phosphate. These findings indicate that the non specific acid phosphatase of the extracts (which catalyzed the hydrolysis of phenyl phosphate) was the only dephosphorylating enzyme that catalyzed the hydrolytic dephosphorylation of the four ribonucleotides, as, if certain specific nucleotidases (which are known to dephosphorylate only nucleotides) were participating in the catalytic process, the variations in the activities of the extracts with the four ribonucleotides will not have been so similar to the variations obtained with phenyl phosphate.

Inability of the extracts to catalyze cleavage of the N-glycosidic linkages of either the four nucleotides or their corresponding nucleosides: Table 1 shows that the extracts could not catalyze cleavage of the N-glycosidic linkages of the four nucleotides, as neither reducing compounds (ribose or ribose 5-phosphate) nor bases could be detected in any of the reaction mixtures. In addition, neither hydrolytic nor phosphorylytic cleavage of N-glycosidic linkages of the corresponding purine and pyrimidine ribonucleosides could be effected by the same extracts over a pH range from 3 to 10. This conclusion was based on inability to detect either ribose or any bases when each of the four ribonucleosides (cytidine, uridine, adenosine and guanosine) was incubated with the extracts in presence and absence of Pi or arsenate at a concentration that represented (on molar bases) about four times that of the substrate.

Separation of the phosphohydrolase from the aminohydrolase using Sephadex G-100 column chromatography: Figure 5 graphically presents the elution diagram of two types of enzymes acid phosphatase and deaminase. It shows two peaks, one of them has an acid phosphatase activity and the other has deaminase activity (Fig.5). Acid phosphatase activity was detected in the fractions from 11-15 with highest specific activity of 85 which corresponds to about 70 fold

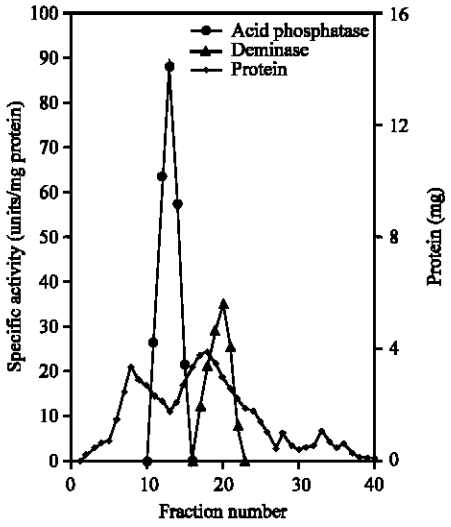


Fig. 5: Separation of the phosphohydrolase from the aminohydrolase using Sephadex G-100 column chromatography

purification while the deaminase was detected in fractions 17-22 and its highest specific activity was about 35 which corresponds to about 30 purification fold.

From the findings recorded during the present investigation and those previously demonstrated concerning extents and pathways of ribonucleotides degradation by the extracts of organisms belonging to the filamentous fungi, it can be stated that, the acid phosphatase of *P. politans* NRC-510 (used in the present study) resembles the acid phosphatases of the two strains of *A. niger* and *A. niger* $NRRL_3$ studied by Elzainy *et al.* (1989) and Ali and Elzainy (2000) in that all of these enzymes could catalyze Pi liberation from the ribonucleotides over a wide range of pH values and this catalytic process was effected by phosphate non-repressible phosphatases. The three extracts could not catalyze cleavage of the N-glycosidic linkages of either the ribonucleotides or the two pyrimidine ribonucleosides. All extracts could hydrolytically deaminate cytidine out of the tested amino bases, their ribonucleosides and ribonucleotides except the extracts of *P. politans* which could deaminate cytidine and adenosine. The cytidine and adenosine deaminase properties of *P. politans* were studied by Elshafei *et al.* (2005a, b). No evidence could be obtained demonstrating participation of specific nucleotidases in dephosphorylation of ribonucleotides by the three extracts.

However, *P. politans* differs from the two *A. niger* strains in that the first organism contained one phosphatase only and not two phosphatases as what has been found in the other two organisms. Also the three organisms differ in that, extracts of *A. niger* strain studied by Elzainy *et al.* (1989) proved to contain a purine nucleoside hydrolase which was active with adenosine and guanosine while extracts of *A. niger* $NRRL_3$ and *P. politans* strains were devoid of this activity. Inorganic phosphate liberation by extracts of *P. politans* at the acidic side of pH was optimum at pH 6 while the analogous catalytic activity of extracts of the two other strains *A. niger* $NRRL_3$ (Ali and Elzainy, 2000) and *A. niger* studied by Elzainy *et al.* (1989) was optimum at pH 2 and pH 4, respectively.

Also on comparing the data obtained on using extracts of *P. politans* with data previously recorded on using extracts of *Penicillium chrysogenum* strain (Elzainy *et al.*, 1979) and *A. terricola* strain (Ali, 2003), it can be seen that extracts of *P. chrysogenum* contained one phosphatase only as what has been found in *P. politans* strain. However *P. chrysogenum* phosphatase was of the alkaline type while that of *P. politans* strain was of the acidic type. In addition extracts of *P. chrysogenum* were suggested to contain both purine and pyrimidine nucleosides hydrolases (Allam *et al.*, 1987), while *A. terricola* contained three phosphate-non repressible enzymes, one acid phosphatase having its optimum activity at pH 4 and two molecular forms of alkaline phatphatases were optimum at pH 8.

Concerning the different ways by which fungal extracts could degrade the ribonuclotides, it appears that these ways include: (1) Hydrolytic cleavage of the phosphate-ester linkages, effected by extracts of the above mentioned fungi. (2) Hydrolytic cleavage of the N-glycosidic linkages of AMP and GMP catalyzed by extracts of *A. niger* (Masaaki and Tomoko, 1978) and of *Neurospora crassa* (Trivedi and Mattoo, 1979). (3) Hydrolytic Cleavage of the amino-purine linkage of AMP, achieved by extracts of *A. oryzae* (Henderson and Paterson, 1973).

ACKNOWLEDGMENT

This study was supported by the National Research Center of Egypt.

REFERENCES

Ali, T.H. and T.A. Elzainy, 2000. Hydrolysis of RNA monomers by extracts of *Aspergillus niger* $NRRL_3$ Antonie van Leeuwenhoek, 77: 229-234.

Ali, T.H., 2003. Existence of an acid phosphatase and two molecular forms of an alkaline phosphatase in *Aspergillus terricola* Egypt. J. Microbiol., 38: 115-126.

Allam, A.M., M.M. Hassan, B.S. Ghanem and T.A. Elzainy 1987. Nature of the enzymes that catalyze the cleavage of the N-glycosidic bond of pyrimidine ribonucleosides in some filamentous fungi. Biochem. Syst. Ecol., 15: 515-517.

Ames, B.N., 1966. Assay of Inorganic Phosphate, Total Phosphate and Phosphatases. Methods in Enzymology. (Colowick, S.P. and N.O. Kaplan, Eds.), Academic Press, New York and London, 8: 115-116.

Armstrong, F.B., 1983. In Biochemistry, 2nd Edn., Oxford University Press, pp: 423-424.

Ashwell, G., 1957. Colorimetric Analysis of Sugars. Methods in Enzymology. Colowick, S.P. and N.O. Kaplan (Eds.), Academic Press, New York and London, 3: 73-74.

Elshafei, A.M., N.H. Ali and L.A. Mohamed, 2005a. Cytidine deaminase activity in *Penicillium politans* NRC-510. J. Basic Microbiol., 45: 335-343.

Elshafei, A.M., L.A. Mohamed and N.H. Ali, 2005b. Deamination of adenosine by extracts of *Penicillium politans* NRC-510. J. Basic Microbiol., 45: 115-124

Elzainy, T.A., Z.A. Elawamry and M.M. Hassan, 1979. Nature and some properties of nucleotide phosphohydrolase from *Penicillium chrysogenum*. Ann. Microbiol., 29: 107-114.

Elzainy, T.A., Z.A. Elawamry and M.M. Hassan and T.H. Aly, 1989. Nucleotide catabolism in *Aspergillus niger*. Acta Microbiol. Pol., 38: 27-36.

Henderson, J.F. and A.R.P. Paterson, 1973. In Nucleotide Metabolism, Academic Press, pp: 152-169.

Layne, E., 1957. Spectrophotometric and turbidimetric methods for measuring protein. Methods Enzymol., 3: 447-451.

Martin, D.W.Jr., 1983. In Harper's Review in Biochemistry. by Martin, D.W. Jr., P.A. Mayes and V.W. Rodwell (Eds.), 19th Edn. pp: 342-343.

Masaaki, K. and F. Tomoko, 1978. Purification and properties of NAD degrading purine nucleosidase from *A. niger*. Can. J. Biochem., 56: 345-348.

Smith, I. and J.W.T. Seakins, 1976. Chromatographic and electrophoretic techniques. William Heinemann Medical Books Ltd., 1: 158-159.

Sutherland, E.W., C.R. Cori, R. Haynes and N.S. Olson, 1949. Purification of the hyperglycemic glycogenolytic factor from insulin and gastric mucosa. J. Biol. Chem., 180: 825-837.

Trivedi, J.P. and A.K. Mattoo, 1979. Environmental factors modulating the levels of nucleosidases in *Neurospora crassa*. Comp. Physiol. Ecol., 4: 286-290.

Vogel, A.I., 1961. Quantitative Inorganic Analysis, Theory and Practice, 3rd Edn. Longmann, Green and Co., London, pp: 783.