J. King Saud Univ., Vol. 8, *Science* (2), pp. 93-106 (A.H. 1416/1996)

The Occurrence and Properties of Dihydrofolate Reductase Isolated from *Pisum sativum*, L. Callus

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(Received 7/11/1992; accepted for publication 2/3/1996)

Abstract. Dihydrofolate reductase (E.C.1.5.1.3) (DHFR) was found in the supernatant fraction of pea callus extract and a number of its properties were investigated. FADH and NADPH were specific requirements for the enzyme reaction. Michaelis constants for FADH2 and NADPH were 1.65×10^{-5} M and 1.98×10^{-4}M, respectively. The optimum pH for the reaction in citrate phosphate buffer was 5.9, in potassium phosphate buffer 6.2 and in Tris – HCl buffer 7.2; the optimum reaction temperature for all buffers used was 30°C. The specific activity of DHFR increased with callus age and coincided with an increase in the DNA and RNA content.

Key words: Callus, dihydrofolate reductase, *Pisum sativum*, L.,

Introduction

Dihydrofolate reductase (E.C.1.5.1.3) (DHFR) is one of the essential enzymes for the methylation cycle of deoxyuridine 5-morphosphate (dUMP) to deoxythymidine 5-monophosphate (dTMP). This enzyme catalyzes the conversion of dihydrofolate (FADH2). The enzyme has been extensively investigated in bacteria [1; 2], mammalian cells [3-5] and protozoa [6-8]. However, in plants, the presence of DHFR has been demonstrated only in pea, soybean and rice, and its properties only partially characterized [9 -12].

There have been no studies involving the isolation and investigation of the properties of this enzyme derived from callus. This general lack of data, together with the advantages of using plant tissue cultures over whole plant parts in such studies [13, p. 66], prompted a detailed investigation of the isolation and characterization of dihydrofolate reductase (DHFR) in pea callus. Rapidly growing cells, in general, require a certain amount of FADH as co-enzyme for the synthesis of thymine. The latter is required in the biosynthesis of DNA for cell division and growth.

Materials and Methods

Plant Source

Pure-bred seeds of field pea (*Pisum sativum*, L. cv Solara) were used. The callus was initiated from epicotyls on a modified [14] medium containing different concentrations of 2,4-D and kinetin. Three media were used containing 10^{-2} M 2,4-D and 10^{-6} M kinetin, 10^{-3} M 2,4-D and 10^{-7} M kinetin or 10^{-4}M 2,4-D and 10^{-8} M kinetin. Callus initiation and growth on such media were as described previously [15]. The callus developed at particular ages was used for extraction and assay of DHFR activity.

Enzyme Extraction and Assay

Dihydrofolate reductase was extracted according to the method of Crosti [12], with modification. Pea callus (0.75 g) was homogenized using a pestle and mortar with 0.1 mM phosphate buffer at pH 7.0 containing 0.56% w/v N-acetylcysteine and the homogenate was disrupted by freezing and thawing. This involved three cycles (10 min each) of freezing in liquid nitrogen and thawing in water bath at 37°C. Trials showed that three cycles of freezing and thawing were sufficient to disrupt callus cells completely. The homogenate was centrifuged at 20,000 X g for 2 h using an MSE-50 superspeed ultracentrifuge.

DHFR activity was assayed spectrophotometrically by the modified methods of Osborn [16]; Mirsa, *et al.* [17] and Crosti [12]. These methods are based on the decrease of absorbance at 340 nm in the presence of NADPH, FADH and the enzyme. Three buffers were used, namely phosphate, citrate and Tris-HCl at concentrations between 10 mM and 60 mM. The reaction mixture contained 50 mM phosphate buffer at pH 7.0, 10.0 mM magnesium sulphate ($MgSO_4 — 7H_2O$), 10 mM 2-mercaptoethanol, 0.1 mM EDTA, 0.08 mM FADH, 0.6 mM NADPH2 and enzyme in a total volume of 3.0 ml. The reaction was initiated by adding 0.1 ml of the enzyme protein (100 $\mu g\ cm^{-3}$). Enzyme activity was determined at 30°C using a Pye-Unicam SP 8000 Spectrophotometer. The amount of protein in the enzyme preparation was determined with Folin-phenol reagent using bovine serum albumin (BSA) as standard. Specific activity was determined as n moles of FAD reduced per min per mg of protein. The enzyme extract was passed through Sephadex G-200 (40 cm × 2.6 cm) for partial purification and molecular weight determination. Standard error of the mean was calculated from 5 different determinations in all estimations.

Nucleic acid extraction and estimation

Nucleic acid content was determined by a method based on the procedure of Cherry [18] as developed by Mohammed & Hassan [19], RNA was determined from the absorption difference at 260 nm and 290 nm. DNA content was estimated using diphenylamine reagent [20].

Molecular weight determination

The molecular weight of the enzyme was determined by the gel filteration method, using Sephadex G-200, according to the general procedure of Andrews [21].

Results

Callus initiation and growth

Three media were used for callus initiation and growth, Murashige and Skoog medium (MS) containing **1)** 2,4-D (10^{-3} M) and kinetin (10^{-7} M), **2)** 2,4-D (10^{-4} M) and kinetin (10^{-8} M) or **3)** 2,4-D (10^{-2} M) and kinetin (10^{-6} M). The results indicated that the optimum medium for support of callus initiation and growth was MS medium containing 2,4-D and kinetin at 10^{-3} M and 10^{-7} M, respectively. The callus developed on this medium was soft, compact and green in color. Callus fresh weight increased markedly with incubation time. No significant increase in callus fresh weight was apparent between 60 and 80 d of incubation (Fig. 1). Therefore, callus of age between 20 and 60 d was used for extraction of DHFR activity.

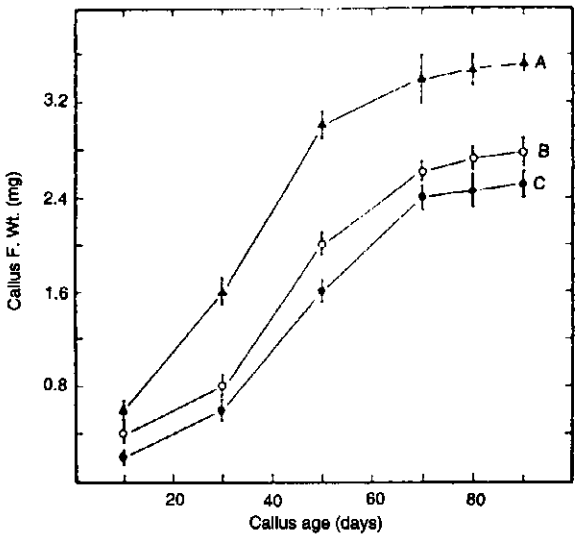

Fig. 1. Changes in fresh weight of pea callus grown at different concentrations of 2,4-D and kinetin for 90 d.

Each point represents the mean of five replicates. Vertical bars represent the standard error of the means.

Callus was grown on media containing 2,4-D (10^{-3} M) and kinetin (10^{-7} M)(A), 2,4-D (10^{-4} M) and kinetin (10^{-8} M) (B) or 2, 4-D (10^{-2} M) and kinetin (10^{-6} M) (C).

Enzyme activity

Initially, the activity of DHFR was measured from 20 d old callus, the highest activity being found in the supernatant fraction (Table 1) which was used in all

Table 1. DHFR activity in the different fractions of pea callus homogenate

Fraction	Activity (OD at 340 mm ^{10}min^{-1})
Homogenate	0.13
Supernatant	0.09
Pellet	0.04

Assay system contained 50 mM potassium phosphate buffer, pH 6.2, 0.08 mM dihydrofolate, 0.6 mM NADPH, 0.1 mM EDTA, 10 mM mercaptoethanol and enzyme in a total volume of 3.0 cm^3

subsequent estimations. The optimum temperature for enzyme activity was found to be 30°C (Fig. 2) and this temperature was used in all subsequent experiments. Optimum

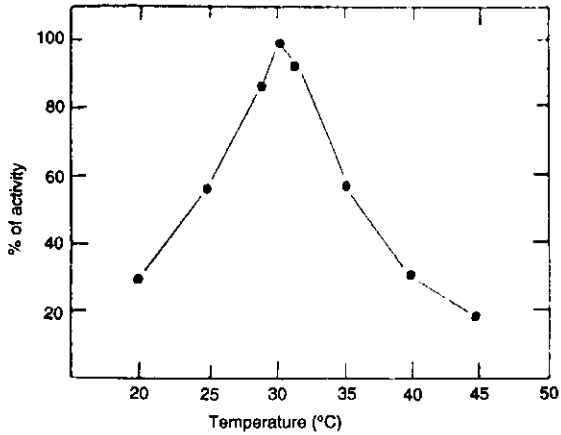

Fig. 2. Effect of temperature on dihydrofolate reductase activity. Standard assay conditions were as described in Table 3.

DHFR activity was measured using 50 mM phosphate, 30 mM Tris-HCl and 30mM citrate buffers (Table 2). The optimum pH values for these buffers are given in Fig. 3. Maximum activity attained in buffers used was between pH 5.9 – 7.2, the optimum pH levels being citrate-phosphate buffer 5.9, potassium phosphate buffer 6.2 and Tris-HCl buffer 7.2. These buffers at particular concentrations and pH were used in all subsequent assays.

Table 2. Specific activity of DHFR, in various concentration of buffers

Concentration (mM)	Buffers		
	Specific activity (nmol FADH min^{-1} mg^{-1} protein)		
	Potassium phosphate	Tris-HCl	Citrate phosphate
10	32.3 ± 1.26	23.0 ± 1.33	20.2 ± 1.03
20	38.3 ± 1.87	37.8 ± 0.89	19.2 ± 0.92
30	39.7 + 2.58	44.4 ± 0.98	22.2 ± 0.84
40	42.3 ± 0.97	32.9 ± 0.87	17.1 ± 0.78
50	48.4 ± 0.99	18.1 ± 0.86	12.9 ± 0.89
60	30.2 ± 1.36	14.8 ± 0.29	0.0 ± 0.00

Each value represents the mean of five separate determinations, ± standard error.

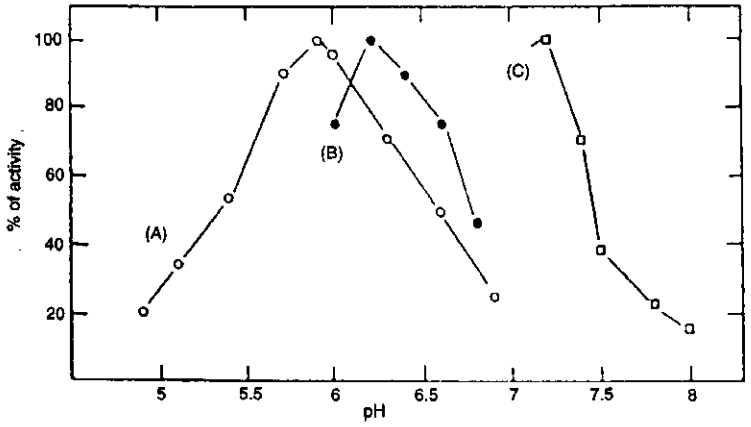

Fig. 3. Effect of pH on pea callus dihydrofolate reductase activity (A) citrate phosphate buffer, (B) potassium phosphate buffer and (C) Tris-HCl buffer.

Properties of DHFR activity

The effect of omission of various components from the assay system are given in Table 3. Omission of NADPH or dihydrofolate greatly reduced enzyme activity, which was not detected in the absence of enzyme under standard conditions.

The effect of Mg^{2+} on enzyme activity is shown in Fig. 4. The activity increased gradually with increased Mg^{2+} concentration up to 10 mM, followed by a decrease thereafter. The effect of other ions (*e.g.* Mn^{2+}, Na^{+}) was also investigated. Mg^{2+} at

Table 3. Effect of assay components on activity of dihydrofolate reductase

Assay conditions	Activity (OD 340 nm ^{10}min^{-1})	% Activity
Complete assay system	0.26	100
Complete assay system without PADH	0.02	8
Complete assay system without NADPH	0.04	15
Complete assay system without enzyme	0.00	0

The complete assay system contained 50 mM potassium phosphate buffer, pH 6.2, 0.08 mM difydrofolate, 0.6 mM NADPH, 1.0 mM EDTA, 10 mM 2-mercaptoethanol and 0.1 ml of the enzyme (100 µg protein) in total volume of 3.0 cm^3.

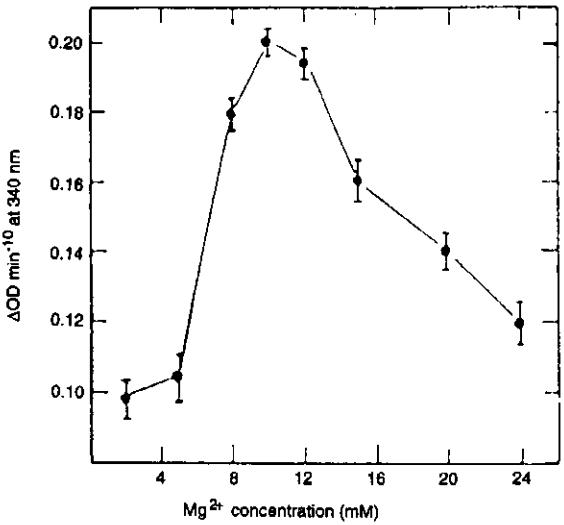

Fig. 4. Effect of Mg^{2+} concentration on dihydrofolate reductase activity. The activity is expressed as the decrease in absorbance at 340 nm^{10} min^{-1} under assay conditions.

(Vertical bars represent the standard error of the mean.)

10 mM was essential for enzyme activity followed by Mn^{2+} and Na^+ (Table 4). The presence of Zn^{2+} in the assay solution, however, completely inhibited enzyme activity.

Reciprocal plots of reaction velocity against substrate or cofactor concentrations were linear for the activities for both FADH and NADPH. The apparent Km values derived from the plots (Figs 5 & 6) were 1.65×10^{-5} M for FADH and 1.98×10^{-4} M for NADPH.

Table 4. Effect of different ions on DHFR activity

Ions (10 mM)	Activity (OD 340 nm ^{10}min^{-1})	% Activity
Mg^{2+}	0.29	100
Mn^{2+}	0.20	69
Na^{+}	0.18	62
Zn^{2+}	0.00	0
Without ions	0.14	48

All ions were at a concentration of 10 mM. DHFR activity was measured under the standard assay conditions given in Table 3.

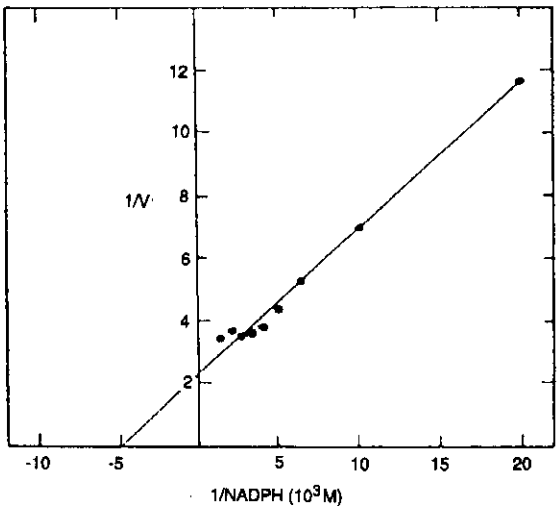

Fig. 5. Effect of various concentrations of NADPH (mM) on dihydrofolate reductase activity of pea callus.

Standard assay conditions are given in Table 3.

From the results above, it would seem that the optimum assay mixture should contain 50 mM phosphate buffer at pH 6.2, 0.1 mM FADH, 0.4 mM NADPH, 10 mM $MgSO_4$, 10 mM 2-mercaptoethanol, 0.1 mM EDTA and 100 ug enzyme protein at a temperature of 30°C. The specific activity of the enzyme under these conditions was 44.5 n mol min^{-1} mg^{-1} of protein as compared with 13.59 n mol min^{-1} mg^{-1} of protein with the initial determination. However, the activity was 164.11 n mol min^{-1} mg^{-1} of protein after

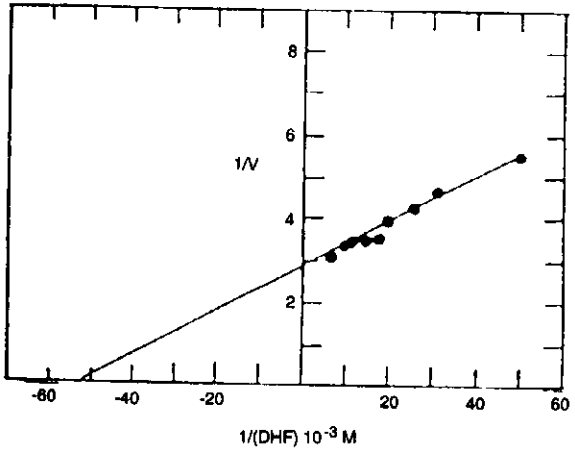

Fig. 6. Effect of various concentrations of 1/FADH (mM) on dihydrofolate reductase activity from pea callus.

passing the extract through Sephadex G-200 (Table 5). Therefore, such conditions were adopted as standard for all subsequent assays.

Table 5. Specific activity of DHFR from pea callus

Conditions used for specific activity determination	Specific activity (n mol FADH min^{-1} mg^{-1} protein)
Preliminary experiments	13.6 ± 0.46
Optimum conditions	44.5 ± 0.89
After passage through Sephadex G-200	164.1 ± 1.67

The activity was measured under the standard conditions described in Table 3.

The molecular weight of the enzyme was determined using Sephadex G-200. It was found that the molecular weight of the DHFR of pea callus was 139,000 (Fig. 7) using cytochrome c, BSA and amylopterin as standards.

Effect of callus age on enzyme activity

The specific activity of DHFR isolated from callus of different ages is given in Table 6. Activity increased with callus age, reaching a maximum at 15 d. No significant differences were apparent between 15 and 19 d old callus. The DNA and RNA content of pea callus followed the same pattern as the enzyme activity (Table 6).

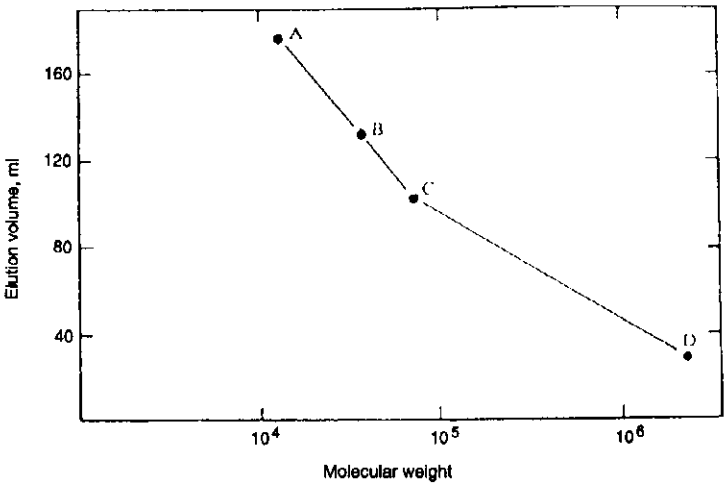

Fig. 7. Determination of molecular weight of dihydrofolate reductase by Sephadex G-200 gel filtration. Marker proteins were cytochrome-c (A), dihydrofolate reductase of pea callus (B), bovine serum albumin (C), and amylopterin (D).

Table 6. Effect of callus age on specific activity of DHFR and the nucleic acid (RNA, DNA) content of pea callus

Callus age after 20 d of subculture	Specific activity	Nucleic acid (mg g^{-1} F.W.)	
		DNA (μg)	RNA (μg)
5	35.4 ± 0.06	12.3 ± 0.01	69.3 ± 0.12
7	38.6 ± 0.06	14.4± 0.01	75.5 ± 0.14
9	41.8 ± 0.06	18.4 ± 0.10	98.3 ± 0.23
11	49.8 ± 0.07	20.3 ± 0.20	109.3 ± 0.65
13	54.6 ± 0.06	21.3 ± 0.01	114.5 ± 0.78
15	57.6 ± 0.06	30.6 ± 0.08	189.4 ± 0.89
17	49.3 ± 0.07	48.4 ± 0.06	213.4 ± 0.99
19	45.0 ± 0.06	50.4 ± 0.07	245.6 ± 0.65
21	41.8 ± 0.06	45.3 ± 0.10	203.7 ± 0.79
23	38.6 ± 0.01	38.4 ± 0.10	189.6 ± 0.96

Each value represents the mean of five separate determinations ± Standard error. Conditions were as described in Table 3.

Discussion

Callus induction and growth appears to be influenced by the nutrient media and culture conditions [22]. The present study showed that growth occurred rapidly in medium containing 2,4-D and kinetin at concentrations of 10^{-3} M and 10^{-7} M respectively. The use of callus instead of tissue from explants has two main advantages. These are that the complex system controlling most of the biochemical activities can be simplified by producing callus and that callus growth can be easily controlled *in vitro*.

The demonstrated persistence of the totipotency of tissue culture is another reason for the increasing use of cell and tissue culture in various biochemical studies [23]. In addition, exogenous factors influencing cultured tissues, such as nutrients, can be readily controlled. The disadvantage of using callus is that it is frequently very heterogeneous. Therefore, total estimations of cellular activities may involve more than one type and developmental stage. Moreover, the use of pea callus is this investigation has certain advantages as in the case of other enzymatic studies [24-27].

The presence of DHFR in pea callus extract may indicate the methylation of dUMP to dTMP in the presence of FADH and NADPH, as has been shown to occur in some animal cells [28]. Moreover, the optimum reaction conditions of the enzyme *in vitro* have been shown to require 50 mM phosphate buffer at pH 6.2, as was also shown in the case of the enzyme isolated from pea seedlings [10]. In addition, the enzyme activity occurred over a wide pH range depending on the buffers used, but DHFR extracted from intact plants showed one absorption peak at optimum pH [10; 11; 29]. The enzyme extracted from pea callus also showed a single peak at the optimum pH used. It differed from that in *E. coli*, however, which has two peaks at pH 4.5 and 7.0 [30] and in human erythrocytes, which peaks at pH 4.6 and 7.6 [31].

These results indicated that the optimum reaction temperature is around 30°C. This was also shown to be true for pea seedlings [10] but differs from the optimum for bacteria and hamster cells, which is around 37°C [32; 33]. The Km values for FADH and NADPH in plants are generally higher than in mammals, bacteria and protozoa. In soybean seedlings, the Km value was reported to be 3.5×10^{-5} M for FADH and 4.15×10^{-15} M for NADPH [11]. In mammalian cells such as pig liver, however, the reported Km was 7.4×10^{-7} M for FADH and 3.2×10^{-6} M for NADPH [34], and in protozoa 5.9×10^{-6} M for FADH and 5.9×10^{-6} M for NADPH [7]. Moreover, in bacteria the value reported was less than in protozoa and mamalian cells [30; 35]. In contrast, the present work showed that the Km values for FADH and NADPH were 1.65×10^{-5} M and 1.98×10^{-4} M respectively. These values were more or less the same as those found in soybean, but differ from the values obtained for other plants, such as rice and carrot [29].

It appears likely that DHFR isolated from pea callus is specific for NADPH, FADH and Mg^{2+} and these results are in agreement with those for DHFR isolated from other living organisms [36-38].

The specific activity of DHFR isolated from pea callus increased with age,

suggesting that callus cells divide rapidly, particularly during the early stages of growth, as indicated by their DNA and RNA content. The increase in DNA content may be correlated with an increase in enzyme activity associated with the biosynthesis of DNA nucleotides during the early stages of callus growth. This supports the suggestion that pea callus depends on *de novo* synthesis of dTMP, since the enzyme thymidine phosphorylase was not detected (data not presented). This observation is in agreement with that of [29] for some trypanosomatids. The results obtained in this study indicate that DHFR isolated from pea callus exhibits the same properties as the enzyme from soybean and rice seedlings. In addition, this enzyme may have an important role in callus growth and in plant growth in general.

References

[1] Blakely, R.L. and McDougall, B.M. " Dihydrofolate Reductase from Streptococcus Faecalis." *Journal of Biological Chemistry*, 234 (1961), 1163-1167.

[2] Ekman, B.T. "Studies on Dihydrofolate Reductase and Trimethoprime Resistance in *E. coli*." *Acta Universitatis Upsaliensis. Abstracts of Uppsala Dissertations from the Faculty of Pharmacy* (1982).

[3] Morales, D.R. and Greenberg, D.M. "Purification and Properties of Dihydrofolate Reductase of Sheep Liver." *Biochemica et biophysica acta*, 85 (1964), 360-376.

[4] Haber, A.D.; Beverley, M.S.; Kiely, L.M. and Sckimke, T.R. "Properties of an Altered Dihydrofolate Reductase Encoded by Amplified Genes in Cultured Mouse Fibroblasts." *Journal of Biological Chemistry*, 256 (1981), 9501-9510.

[5] Rao, G.N. and Cotlier, E. "The Enzymatic Activities of GTP Cyclohydrolase, Sepiapterin Reductase, Dihydropteridine Reductase and Reductase and Tetrahydrobiopterin Content in Mammalian Ocular Tissue and Human Senile Cataracts." *Comparative Biochemistry and Physiology*, 80 B (1985), 61-66.

[6] Jaffe, J.J.; McCormack, J.J. and Gutterdige, W.E. "Comparative Study of Dihydrofolate Reductases within the Genus Trypanosoma." *Experimental Parasitology*, 25 (1969), 311-318.

[7] Jaffe, J.J. "Dihydrofolate Reductase from Filarial Worms and Schistosomes." *Annals of the Academy of Science, New York*, 186 (1971), 113-114.

[8] Coderre, A.J.; Beverley, M.S.; Schimke, T.R. and Santi, V.D. "Over Production of Bifunctional Thymidylate Synthetase, Dihydrofolate Reductase and DNA Amplification in Methotrexate Resistant *Leishmania tropica*." *Proceedings of the Natural Academy of Science, USA*, 80 (1983), 2132-2136.

[9] Suzuki, N. and Iwai, K. "The Occurrence and Properties of Dihydrofolate Reductase in Pea Seedling." *Plant and Cell Physiology*, 11 (1970), 199-208.

[10] ReddY, A. and Rao, N.A. "Dihydrofolate Reductase from Soybean Seedlings: Characterization of the Enzyme Purified by Affinity Chromatography." *Archives of Biochemistry and Biophysics*, 174 (1976), 657-683.

[11] ————"Dihydrofolate Reductase from Soybean Seedlings: A Fluorescence and Circular Dichroism Study," *Ibid*, 183 (1979), 11475-11484.

[12] Crosti, P. "Effect of Folate Analogues on the Activity of Dihydrofolate Reductase and on the Growth of Plant Organisms." *Journal of Experimental Botany*, 32 (1981), 717-723.

[13] Overton, K.H. "Biosynthesis of Mevalonic Derived Compounds in Cell Cultures." In: *Plant Tissue Culture and Its Biotechnological Application*,. W. Basz, E. Reinhard and M. H. Zenk. (Eds). New York: Springer Verlag, 1977.

[14] Murashige, T. and Skoog, L. "A Revised Medium for Rapid Growth and Bioassays with Tobacco Tissue Cultures." *Physiologia Plantarum*, 15 (1962), 473-497.

[15] El Sayed, H. and Kirkwood, R.c. "Solute Accumulation in Soybean (*Glycine max*, L.) Cells Adapted to NaCl Salinity." *Phyton Journal*, 32, No. 2 (1992), 233-249.

[16] Osborn, M.J. and Huennekes, F.M. "Enzymatic Reduction of Dihydrofolic Acid." *Journal of Biological Chemistry*, 233 (1958), 969-974.

[17] Misra, K.S.R.; Humphrey, M.; Friedkin, A.; Goldin, A. and Crawford, E.J. "Increased Dihydrofolate Reductase Activity as a Possible Basis of Drug Resistance in Leukaemia." *Nature*, 189 (1961), 39.

[18] Cherry, J.H. "Nucleic Acid Determination in Storage Tissue of Higher Plants." *Plant Physiology*, 37 (1962), 670-678.

[19] Mohammad, A.M.S. and Hassan, H.A. "Effect of Standard and Prospective Growth Regulators on Sunflower Callus." II- Changes in Protein, RND, DNA and Carbohydrate Content." *Journal of the University of Kuwait (Science)*, 85 (1988), 69-77.

[20] Giles, K.W. and Mayer, A. "An Important Diphenylamine Reagent for Estimation of DNA Concentration." *Nature*, 20 (1965), 93.

[21] Andrews, P. "The Gel Filtration Behavior of Proteins Related to Their Molecular Weight Over a Wide Range." *Biochemistry Journal*, 96 (1965), 595-600.

[22] Street, H.S. *Plant Tissue and Cell Culture*. Oxford: Blackwell Scientific Publication, 1978.

[23] Barwale, U.B.; Kerns, H.R. and Widholm, J.M. "Plant Regeneration from Callus Cultures of Several Soybean Genotypes via Embryogenesis and Organogenesis." *Planta*, 167 (1986), 473-481.

[24] Jaspars, E.M.J. and Veldstra, H. "An Amylase from Tobacco Crown Gall Tissue Cultures. I - Purification and Some Properties of the Enzyme Pattern of Amylase Iso-enzymes in Different Tobacco Tissue." *Physiologia Plantarum*, 18 (1965), 604-625.

[25] Dougall, D.K. "Threonine Deaminase from Paul's Scarlet Rose Tissue Cultures." *Phytochemistry*, 9 (1970), 959-964.

[26] Goh, C.J. "Respiratory Enzyme Systems in Cultured Callus Tissue." *New Phytologist*, 70 (1971), 753-755.

[27] Kanamori, I. and Ashihara, H. "Pyrimidine Nucleotid Biosynthesis in *Vinca rosea* Cells. Changes in the Activity of *de novo* and Salvage Pathways During Growth in Suspension Culture." *Journal of Experimental Botany*, 32 (1981), 68-78.

[28] Al Chalabi, K. and Gutteridge, W.E. "Catabolism of Deoxythymidylate in Some Trypanosomatids." *Parasitology*, 74 (1977), 299-312.

[29] Cella, R.; Crosti, P.; Nielsen, E. and Parisi, B. "Biochemical Basis of Different Sensitivity to Methotrexate in *Daucus carota* and *Oryza sativa* Cell Cultures." *Journal of Experimental Botany*, 34 (1983), 1189-1195.

[30] Baccanari, D.; Phillips, A.; Smith, S.; Sinski, D. and Burchall, J. "Purification and Properties of *E. coli*, Dihydrofolate Reductase." *Biochemistry*, 14 (1975), 5267.

[31] Bertino, JR.; Cashmore, A.R. and Hillcoat, B.L. "Induction of Dihydrofolate Reductase: Purification and Properties of the Induced Human Erythrocyte and Leukocyte Enzyme and Normal Bone Marrow Enzyme." *Cancer Research*, 30 (1970), 2372-2378.

[32] Hillcoat, L.B. and Blakely, L.R. "Dihydrofolate Reductase of *Streptococcus faecalis*. I - Purification and Some Properties of Reductase from the Wild Strain and from Strain A." *Ibid.* 241 (1966), 2995-3001.

[33] Hanggi, U.J. and Littlefield, J.W. "Isolation and Characterization of the Multiple Forms of Dihydrofolate Reductase from Methotrexate Resistant Hamster Cells." *Journal of Biological Chemistry*, 249 (1974), 1390-1397.

[34] Smith, S.L.; Patrick, P.; Stone, D.; Phillips, A.W. and Burchall, J.J. "Porcine Liver Dihydrofolate Reductase." *Journal of Biological Chemistry*, 254 (1979), 11475-11484.

[35] Burchall, J.J. and Hitching, G.H. "Microbial Dihydrofolate Reductase." *Federation Proceedings*, 23 (1964), 429.

[36] Gutteridge, W.E.; Jaffe, J.J. and McCormack, J.J. "The Gel Filtration Behavior of Dihydrofolate Reductase from Culture forms of Trypanosomatids." *Biochimica et biophysica acta*, 191 (1969), 753-755.

[37] Gutteridge, W.E.; Ogilvie, B.M. and Dunnett, S.J. "Presence and Properties of a Dihydrofolate Reductase from a Parasitic Nematode, Nippostronglylus Brasiliensis." *International Journal of Biochemistry*, 1 (1970), 230.

[38] Platzer, E.G. "Dihydrofolate Reductase in *Plasmodium iophurae* and Duckling Erythrocytes." *Journal of Protozoology*, 2 (1974), 400-405.

خواص وصفات داي هيدروفولات ريداكتاز المعزول من كالس نبات البسلة

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(سُلِّم في ١٣ جمادى الأولى ١٤١٣هـ؛ وقُبل للنشر في ١٣ شوال ١٤١٦هـ)

ملخص البحث. تمَّ فحص عدد من صفات وخواص داي هيدروفولات ريداكتاز (DHFR) المجزّء في الرشيح للكالس المستخلص من نبات البسلة، وجد أن NADPH2 & FADH2 متخصصين في احتياجات التفاعل الأنزيمي. أيضًا وجد أن ثابت ميخاليل لكل من NADPH2 & FADH2 هو ١,٦٥ × ١٠^-٥ مولار و١,٩٨ × ١٠^-٥مولار بالترتيب. كما لوحظ أن درجة أيون الهيدروجين المثل لتفاعل المحلول المنظم من سترات الفوسفات هي ٥,٩. أما في حالة HCL - Tris فالمحلول المنظم كان ٧,٢. أيضًا لوحظ أن درجة الحرارة المثل لتفاعل كل من المحاليل المنظمة كانت عند ٣٠°م. أما بالنسبة لنشاط HDFR المتخصص قد ازدادت مع زيادة عمر الكالس وأيضًا طابقت هذه الزيادة مع محتويات كل من DNA & RNA.