Proteases in egg, miracidium and adult of Fasciola gigantica. Characterization of serine and cysteine proteases from adult

Abstract:
Proteolytic activity of 0–12 day old eggs, miracidium and adult worm of Fasciola gigantica was assessed and proteases were partially purified by DEAE-Sepharose and CM-cellulose columns. Four forms of protease were separated, Pla, Plb, Plc and PII. Purifications were completed for Plc and PII using Sephacryl S-200 chromatography. A number of natural and synthetic proteins were tested as substrates for F. gigantica Plc and PII. The two proteases had moderate activity levels toward azoalbumin and casein compared to azocasein, while gelatin, hemoglobin, albumin and fibrin had very low affinity toward the two enzymes. Amidolytic substrates are more specific to protease activity. Plc had higher affinity toward BAPNA– HCl (N-benzoyl–arginine–p-nitroanilide–HCl) and BTPNA– HCl (N-benzoyl–tyrosine–p-nitroanilide–HCl) at pH 8.0 indicating that the enzyme was a serine protease. However, PII had higher affinity toward BAPNA at pH 6.5 in the presence of sulfhydryl groups (h-mercaptoethanol) indicating that the enzyme was a cysteine protease. The effect of specific protease inhibitors on these enzymes was studied. The results confirmed that proteases Plc and PII could be serine and cysteine proteases, respectively. The molecular weights of F. gigantica Plc and PII were 60,000 and 25,000, respectively. F. gigantica Plc and PII had pH optima at 7.5 and 5.5 and K\textsubscript{M} of 2 and 5 mg azocasein/mL, respectively. For amidolytic substrates, Plc had K\textsubscript{M} of 0.3 mM BAPNA/mL and 0.5 mM BTPNA/mL at pH 8.0 and PII had K\textsubscript{M} of 0.6 mM BAPNA/mL at pH 6.5 with reducing agent. F. gigantica Plc and PII had the same optimum temperature at 50°C and were stable up to 40°C. All examined metal cations tested had inhibitory effects toward the two enzymes. From
substrate specificity and protease inhibitor studies, Plc and PII could be designated as serine and cysteine PII, respectively.

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Keywords: F. gigantica; Development; Cysteine protease; Serine protease; Purification; Characterization
Urea cycle of Fasciola gigantica: Purification and characterization of arginase

Abstract:

The ornithine – urea cycle has been investigated in Fasciola gigantica. Agrinase had very high activity compared to the other enzymes. Carbamoyl phosphate synthetase and ornithine carbamoyltransferase had very low activity. A moderate enzymatic activity was recorded for argininosuccinate synthetase and argininosuccinate lyase. The low levels of F. gigantica urea cycle enzymes except to the arginase suggest the urea cycle is operative but its role is of a minor important. The high level of arginase activity may benefit for the hydrolysis of the exogenous arginine to ornithine and urea. Two arginases Arg I and Arg II were separated by DEAE-Sepharose column. Further purification was restricted to Arg II with highest activity. The molecular weight of Arg II, as determined by gel filtration and SDS-PAGE, was 92,000.

The enzyme was capable to hydrolyze l-arginine and to less extent l-canavanine at arginase: canavanase ratio (> 10). The enzyme exhibited a maximal activity at pH 9.5 and K_m of 6 mM. The optimum temperature of F. gigantica Arg II was 40°C and the enzyme was stable up to 30°C and retained 80% of its activity after incubation at 40°C for 15 min and lost all of its activity at 50°C. The order of effectiveness of amino acids as inhibitors of enzyme was found to be lysine > isoleucine > ornithine > valine > leucine > proline with 67%, 43%, 31%, 25%, 23% and inhibition, respectively. The enzyme was activated with Mn2+, where the other metals Fe2+, Ca2+, Hg2+, Ni2+, Co2+ and Mg2+ had inhibitory effects.

Keywords: Fasciola gigantica; Urea cycle; Arginase; Purification; Characterization; Amino acids
Genetic variability and relationships among Egyptian Cowpea cultivars

Abstract:
Haptoglobin Gene Polymorphism in Type 2 Diabetic Patients with and without Nephropathy

Abstract

Background: The development and progression of diabetic microvascular complications including nephropathy are related to the degree of glycemic control and oxidative stress and may be influenced by genetic factors. The aim of the present study was to investigate the association between haptoglobin (Hp) gene polymorphism and the occurrence of diabetic nephropathy in patients with type 2 diabetes mellitus and to find a possible link between Hp phenotypes and the inflammatory parameters; serum C-reactive protein (CRP), interleukin-6 (IL-6), and Hp.

Methods: The study included 60 normotensive type 2 diabetic patients (N5 years duration) categorized into three equal groups (normo-, micro-, and macroalbuminuric), according to urinary albumin excretion (UAE). In addition, 20 age- and sex-matched individuals were selected to serve as a control group. Serum CRP, IL-6, and Hp concentrations were measured and Hp phenotyping was conducted using polyacrylamide gel electrophoresis.

Results: The frequency of Hp phenotype 1-1 (Hp 1-1) in diabetic patients with normoalbuminuria was 7/20 (35%) as compared with 1/20 in diabetics with macroalbuminuria (p= 0.02). However, the frequency of Hp 2-2 was greater in diabetics with macroalbuminuria (12/20, 0.06%) than in those with normoalbuminuria or controls (5/20, 25%; p= 0.03). Patients with diabetic nephropathy (micro- or macroalbuminuria) had higher levels of serum CRP, IL-6, and Hp than those without nephropathy (normoalbuminuria). Serum Hp levels in type 2 diabetics were higher in Hp phenotype 2-2 than in Hp 1-1; however, serum CRP and IL-6 levels did not differ significantly between Hp phenotype groups. Moreover, there were significant positive correlations between UAE and serum levels of CRP, IL-6, and Hp in diabetic...
Conclusions: Hp phenotype 2-2 is considered to be a major susceptibility gene for the development of nephropathy in type 2 diabetic patients. In addition, the significant association between inflammatory parameters and UAE indicates that inflammation may be a pathogenic mechanism of renal injury in type 2 diabetics. Moreover, serum IL-6 and Hp may be good prognostic factors for the development of nephropathy in the course of diabetes mellitus. Future research on the use of anti-inflammatory therapy may result in a new approach to the treatment and prevention of diabetic nephropathy.

Keywords: Diabetic nephropathy; Oxidative stress; Inflammation; Haptoglobin; Interleukin-6; Susceptibility genes
Yucca schidigera” Extraction in broiler chicken under ammonia stress

Abstract
Clinical significance of serum levels of matrix metalloproteinase-9 (MMP-9), tissue inhibitor of metalloproteinase-1 (TIMP-1) and their molar ratio in patients with bronchial asthma and chronic obstructive pulmonary disease

Abstract
Is there any role for statins in management of osteoporosis and dementia

Abstract
Cod liver oil in chemically induced Diabetes mellitus in rats

Abstract

Alloxan induces diabetes in experimental animals through the selective damage of pancreatic cells. Cod liver oil (CLO) is an important source of long-chain \(-3\) fatty acids (eicosapentaenoic and docosahexaenoic acids) and vitamins A, E, and D. In the present study, the possible protective effect of CLO against alloxan-inducing diabetes was investigated in rats. Sixty male albino rats were divided into six groups (ten rats each) as following: Group I (control group), rats fed on a standard diet; Group II (diabetic group), rats injected intraperitoneally with alloxan (75 mg kg\(^{-1}\) day\(^{-1}\)) for five consecutive days; Group III (CLO group), rats received orally 100 \(\mu\)l of CLO for five consecutive days; Group IV (treated group), rats injected with alloxan for five consecutive days followed by CLO administration for five consecutive days; Group V (protected group) rats received CLO for five consecutive days followed by alloxan injection for five consecutive days, and Group VI (simultaneous group), rats received CLO and alloxan at the same time for five consecutive days. After 30 days from starting the injection, plasma glucose, insulin, tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)), interleukine-6 (IL-6), and nitric oxide (NO) were investigated. Results showed that plasma levels of glucose, TNF-\(\alpha\), and IL-6 were significantly elevated, while levels of plasma NO and insulin were significantly decreased in diabetic rats when compared with the control group. Oral administration of CLO (protected and simultaneous groups) ameliorated the deleterious effects of alloxan by lowering glucose, TNF-\(\alpha\), IL-6 and by slightly elevating plasma insulin and NO levels. It is concluded that CLO might prevent alloxan action by suppressing the release of inflammatory cytokines (TNF-\(\alpha\) and IL-6) that are involved in \(\beta\)-cell damage and development of diabetes.

Key words: cod liver oil, alloxan, inflammatory cytokines, rat