Human acylation stimulating protein enhances triacylglycerol biosynthesis in plant microsomes

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Abstract Diacylglycerol acyltransferase has a universal role in catalyzing the acyl-CoA-dependent formation of triacylglycerol in microorganisms, animals and plants. Acylation stimulating protein, from human blood, is known to enhance diacylglycerol acyltransferase activity and triacylglycerol biosynthesis in human adipocytes. In the current study, acylation stimulating protein was also shown to enhance diacylglycerol acyltransferase activity in microsomes from cell suspension cultures of oilseed rape. Enzyme stimulation occurred over the pH range of 6–9 but the degree of stimulation decreased with increasing ionic strength at pH 7.4. Varying acyl-CoA concentration did not affect the degree of stimulation. Membranes from triacylglycerol producing cells in plants and humans may have similar binding sites for acylation stimulating protein which have been preserved during molecular evolution. The results suggest that human acylation stimulating protein may be useful in modifying lipid biosynthesis in plants.

Key words: Diacylglycerol acyltransferase; Acylation stimulating protein; Microspore-derived culture; *Brassica*

1. Introduction

The Kennedy or *sn*-glycerol-3-phosphate pathway is the major route for the biosynthesis of triacylglycerol (TG) in membranes from many microorganisms, plants and animals [1–3]. The pathway involves the sequential acylation of the glycerol backbone beginning with *sn*-glycerol-3-phosphate. The phosphate group is removed prior to acylation at the *sn*-3 position. Acylation of *sn*-1,2-diacylglycerol (DG) is catalyzed by the acyl-CoA-dependent diacylglycerol acyltransferase (DGAT, EC 2.3.1.20). This enzyme reaction may limit the flow of carbon into TG in both mammalian [4,5] and plant [6,7] systems.

In human adipocytes, TG biosynthesis is enhanced through the action of a small molecular mass (8932 Da) basic protein (pI = 9.1) known as acylation stimulating protein (ASP) [8,9]. ASP is produced through the action of adipsin and factor B, on complement C3, all of which are synthesized and secreted by human adipocytes [9–12]. Carboxypeptidase N catalyzes the removal of a terminal arginine residue from the precursor fragment C3a to generate ASP. The effect of ASP on promoting TG biosynthesis in human adipocytes involves a signal transduction pathway, beginning at the cell surface, which is mediated through protein kinase C [8,13]. In this pathway, DGAT may be covalently modified to produce a more active form of the enzyme. As well, an earlier study demonstrated that ASP could stimulate DGAT activity in microsomes from human adipose tissue by about 2-fold [14]. ASP did not affect microsomal phosphatidate phosphatase activity whereas *sn*-glycerol-3-phosphate acyltransferase activity was stimulated by only 23%. Studies of human subjects have shown that plasma levels of ASP are considerably higher in obese individuals [15]. ASP has been identified in a number of different mammalian species and comparisons of the protein between species have indicated 67–72% primary sequence homology [16].

Microspore-derived cell suspension cultures of oilseed rape (*Brassica napus* L. cv Jet Neuf) have been shown to produce TG [17–19]. Unlike zygotic embryos from developing seeds, the cell suspension culture produces TG in the absence of cellular differentiation [19]. Recently, a number of non-protein factors were identified that enhanced DGAT activity in microsomes from the cell suspension cultures [20]. In the current study, we demonstrate that human ASP also stimulates DGAT activity in microsomes prepared from the cell suspension cultures.

2. Materials and methods

2.1. Chemicals and biochemicals

[1-^{14}C]Oleic acid (51 Ci mol^{-1}) was from Amersham Canada Ltd., Oakville, Ont., Canada). DG was from Avanti Polar Lipids, Ltd. (Alabaster, AL, USA). [1-^{14}C]Oleoyl-CoA was prepared from radiolabeled oleic acid and CoA using acyl-CoA synthetase [21]. ASP was purified as described previously [13]. High-performance liquid chromatography grade solvents were from BDH, Inc. (Toronto, Ont., Canada). All other biochemicals used were of the highest purity available from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

2.2. Preparation of plant microsomes

Microspore-derived cell suspension cultures of *B. napus* L. cv Jet Neuf were maintained with some modifications [20] to a previously described protocol [22]. Microsomes (10 000–100 000 × g pellet) were prepared from cell homogenate according to Byers et al. [20]. Microsomal protein content was determined using the Bio-Rad protein microassay based on the Bradford procedure [23], with bovine serum albumin as the standard.

2.3. Assay of DGAT activity

DGAT activity was assayed under similar conditions to those described previously [20]. The standard reaction mixture contained 0.2 M

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HEPES–NaOH (pH 7.4), 0.5 mM $MgCl_2$, 330 μM *sn*-1,2-dioleoylglycerol, 15 μM [1-^{14}C]oleoyl-CoA (51 Ci mol^{-1}), 0.1% Tween-20, 6 mg bovine serum albumin ml^{-1} and 5 μl of resuspended microsomes containing 28–32 μg protein. ASP was preincubated with microsomes and other reaction mixture components, except acyl-CoA, for a period of 30 min at 30°C. Enzyme reactions, which were also conducted at 30°C, were initiated with 5 μl of 180 μM [1-^{14}C]oleoyl-CoA and allowed to proceed for 10 min unless indicated otherwise. Control (−ASP) and test (+ASP) reactions contained equal concentrations of bovine serum albumin. The reaction was terminated with 10 μl of 5% (w/v) sodium dodecyl sulfate. A 50 μl aliquot of each reaction mixture was spotted onto a Merck silica gel 60 H (VWR Canlab, Mississauga, Ont., Canada) preparative TLC plate (set up to accommodate seven samples) along with a trioleoylglycerol standard in the middle lane. After allowing the spots to dry, the plates were developed with hexane/diethyl ether (80:20, v/v). Sections of silica containing TG from each lane were scraped into scintillation vials, combined with 5 ml Ecolite™ (+) scintillant (ICN Biomedicals, Inc., Irvine, CA, USA) and assayed for radioactivity.

3. Results and discussion

A time course assay for the production of TG catalyzed by plant microsomal DGAT in the absence or presence of ASP is shown in Fig. 1. The results indicated that human ASP stimulated TG production over a period of 60 min. Further tests on stimulation, however, were based on a 10 min assay. The phosphate-buffered saline used for storing ASP [13] and the enzyme reaction buffer [20] both contained bovine serum albumin (1 mg/ml in storage buffer and 6 mg/ml in reaction mixture) to minimize non-specific losses of the stimulatory protein. It was also essential to store ASP at −80°C in silanized tubes to prevent aggregation and adsorption to surfaces [8,13]. ASP was frozen and thawed twice prior to use in our experiments. The effect of various concentrations of ASP on stimulation of DGAT activity is depicted in Fig. 2. Enzyme activity was stimulated by 50% at 100 μg ASP/ml (11 μM) and over 100% at 400 μg ASP/ml. Previously, an ASP concentration of 30 μg ASP/ml was shown to stimulate DGAT activity by 50% in microsomes from human adipocytes [14].

The effect of pH was examined in order to develop some insight into the mechanism of stimulation of TG production in plant microsomes. The dependence of microsomal DGAT activity on pH (Fig. 3) was similar to that previously reported for membrane-bound [24] and solubilized [25] DGAT from microspore-derived embryos of oilseed rape. ASP stimulated DGAT activity from pH 6 to 9 with maximum stimulation occurring at neutral pH in the presence of HEPES–NaOH buffer (Fig. 3). Stimulation was not as great in the presence of phosphate buffer. When assessed at pH 7.5, the conductivity of the phosphate buffer was over 4-fold greater than that of HEPES–NaOH buffer suggesting that stimulation was reduced at higher ionic strength.

The effect of ionic strength on stimulation was examined by introducing increasing concentrations of KCl into the standard reaction mixture at pH 7.4 (Fig. 4). Concentrations of 50 and 100 mM KCl stimulated control DGAT activity but

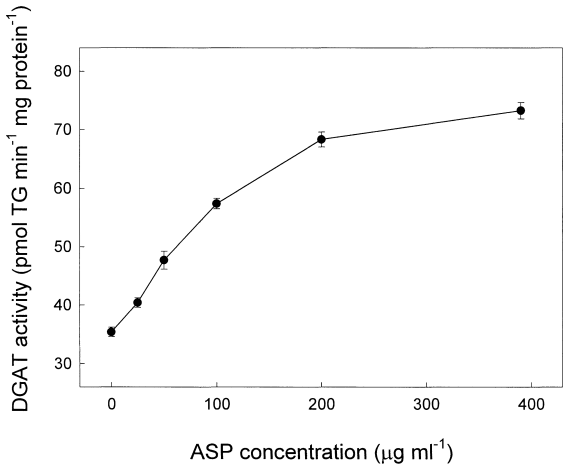

Fig. 2. Effect of ASP concentration on DGAT activity in plant microsomes. Twenty-eight micrograms of microsomal protein were used per reaction mixture. Each data point represents the mean of triplicate assays ± S.E.M.

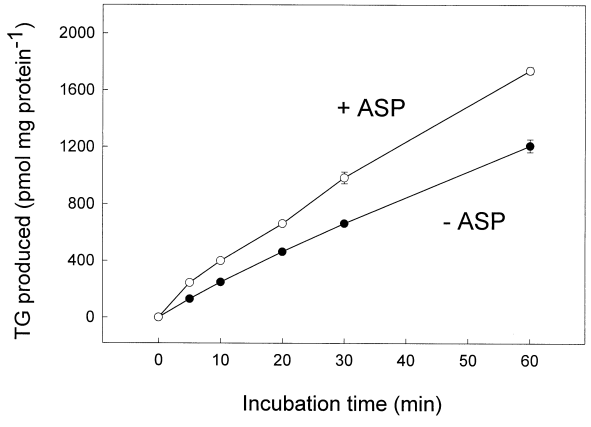

Fig. 1. Time course assay for the production of TG catalyzed by plant microsomal DGAT in absence or presence of ASP (200 μg ml^{-1}). Thirty-two micrograms of microsomal protein were used per reaction mixture. Each data point represents the mean of triplicate reactions ± S.E.M. The rate at which TG production increased with time differed between treatments (i.e. in the presence or absence of ASP; ANOVA, overall $R^2 = 0.994$, time × ASP $f_{1/26} = 101.3$, $P < 0.0001$).

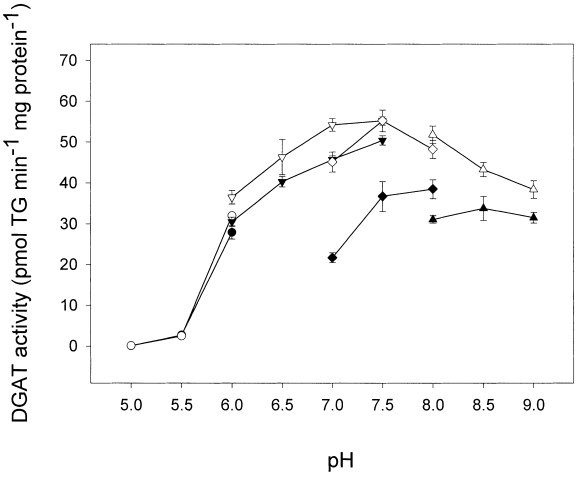

Fig. 3. Effect of pH on plant microsomal DGAT activity in the absence or presence of ASP (200 μg ml^{-1}). Buffer species were used at a total concentration of 0.2 M. Filled symbols indicate assays without ASP. Open symbols indicate assays with ASP. Circle, succinic acid–NaOH; inverted triangle, potassium phosphate buffer; diamond, HEPES–NaOH; triangle, Bicine–NaOH. Thirty micrograms of microsomal protein were used per reaction mixture. Each data point represents the mean of triplicate assays ± S.E.M.

the effect was diminished at higher concentrations. The response of microsomal DGAT activity to increasing KCl concentration was similar to that of DGAT activity in microsomes from microspore-derived embryos of oilseed rape assayed at various concentrations of NaCl [25]. Increasing concentrations of KCl resulted in decreased ASP-dependent stimulation of DGAT activity with complete abolition of stimulation at a concentration of 200 mM KCl. The ionic strength effect on DGAT stimulation suggested that the interaction between ASP and its binding site in the microsome involves charge–charge interactions and/or that increased KCl concentration may result in conformational changes that reduce the binding strength of the interacting species.

The degree of stimulation of plant DGAT activity by human ASP remained constant over a range of acyl-CoA concentrations (5–30 μM) (Fig. 5). These data suggested that ASP did not improve substrate delivery to the active site of plant DGAT. A similar conclusion was drawn based on the effect of acyl-CoA concentration on the stimulation by ASP of microsomal DGAT activity from human adipocytes [14]. Exogenous DG has been shown to have little effect on the activity of microsomal plant DGAT under the assay conditions [20]. Presumably, the microsomes contained sufficient DG to support most of the enzyme activity [20,25]. Therefore, the effect of varying the DG content of the reaction mixture in the absence or presence of ASP was not examined.

There may be similar binding sites for ASP in TG forming cells of both mammals and plants. Although human ASP has been shown to stimulate TG biosynthesis in adipocytes via the protein kinase C pathway [13], there is some evidence to suggest that ASP may interact directly with human DGAT [14]. The putative interaction of ASP with mammalian DGAT and plant DGAT would likely involve conserved amino acid sequences in these enzymes. Indeed, a recent study has demonstrated that the predicted amino acid sequences of mouse DGAT [26] and oilseed rape DGAT share about 40% identity [19,27,28]. No sequences homologous to ASP were identified in plants in non-redundant and expressed sequence tag databases using either the human synthetic C3a (Accession No. M65080), mouse complement C3 precursor (Accession No. P01027) or the pig complement C3a (Accession No. P01025)

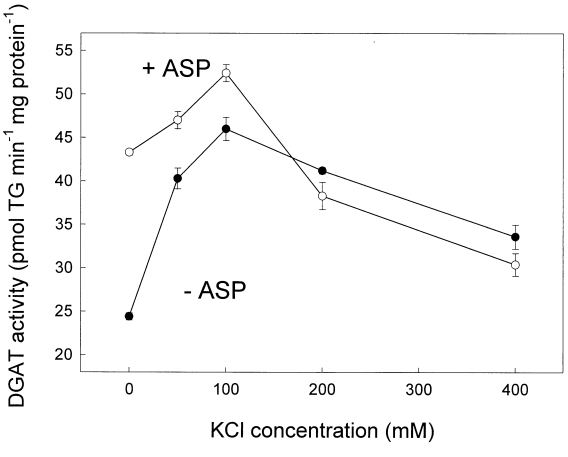

Fig. 4. Effect of KCl concentration on plant microsomal DGAT activity in the absence or presence of ASP (200 μg ml^{-1}). Thirty-two micrograms of microsomal protein were used per reaction mixture. Each data point represents the mean of triplicate assays ± S.E.M.

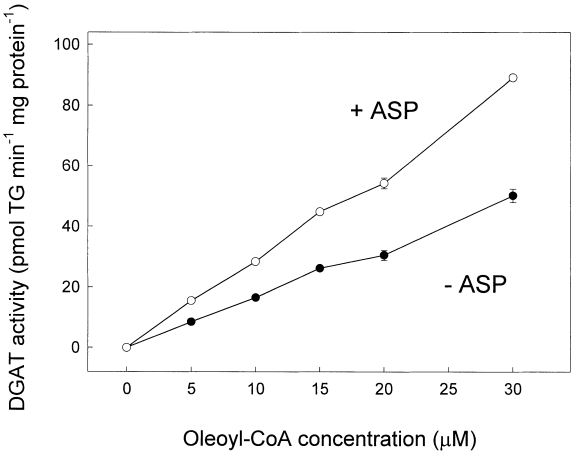

Fig. 5. Effect of oleoyl-CoA concentration on plant microsomal DGAT activity in the absence or presence of ASP (200 μg ml^{-1}). Thirty-two micrograms of microsomal protein were used per reaction mixture. Each data point represents the mean of triplicate assays ± S.E.M.

sequences. These results suggest that sequences homologous to human ASP are absent in plants or, alternatively, have not as yet been identified in plants. Binding studies with purified or recombinant plant DGAT would be necessary to unequivocally establish that ASP interacts directly with the acyltransferase. Alternatively, ASP may exert other effects on the plant membrane that indirectly affect DGAT activity. ASP could prove to be a useful tool in further examining relationships in TG biosynthesis between plants and animals. As well, from the perspective of metabolic engineering, expression of ASP in plants may result in modulation of storage lipid accumulation.

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