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ARTICLE *in* INTERNATIONAL JOURNAL OF BIOLOGICAL MACROMOLECULES · APRIL 2006

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Angiotensin converting enzyme inhibitory activity of amino acid esters of carbohydrates

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Received 8 November 2005; received in revised form 18 January 2006; accepted 19 January 2006
Available online 10 March 2006

Abstract

L-Alanyl-D-glucose, L-valyl-D-glucose, L-phenylalanyl-D-glucose and L-phenylalanyl-lactose esters were synthesized enzymatically using two lipases viz., *Rhizomucor miehei* lipase (RML) and porcine pancreas lipase (PPL) and tested for their potential as inhibitors of angiotensin converting enzyme (ACE) in vitro. The esters exhibited concentration related ACE inhibitory activity. The potency of the various esters measured in terms of IC_{50} values were as follows: L-phenylalanyl-D-glucose, IC_{50}-0.121 mM (mixture of five diastereomeric esters: 6-*O*-24.1%; 3-*O*-23.3%; 2-*O*-19.2%; 2,6-di-*O*-16.6% and 3,6-di-*O*-16.8% from the total yield of 92.4%); L-phenylalanyl-lactose, IC_{50}-0.229 mM (mixture of three diastereomeric esters: 6-*O*-42.1%; 6′-*O*-30.9%; and 6,6′-di-*O*-27.0% from the total yield of 50.58%); alanyl-D-glucose, IC_{50}-0.23 mM (mixture of five diastereomeric esters: 6-*O*-46.7%; 3-*O*-11.5%; 2-*O*-19.9%; 2,6-di-*O*-6.6% and 3,6-di-*O*-15.3% from the total yield of 26.5%) and L-valyl-D-glucose, IC_{50}-0.396 mM (mixture of five diastereomeric esters: 6-*O*-32.4%; 3-*O*-26.5%; 2-*O*-26.4%; 2,6-di-*O*-8.8% and 3,6-di-*O*-5.9% from the total yield of 68.2%). These in vitro data suggest a potential therapeutic role for the aminoesters of carbohydrates as inhibibitors of ACE.

Keywords: Angiotensin converting enzyme inhibitor; L-Alanyl-D-glucose; L-Valyl-D-glucose; L-Phenylalanyl-D-glucose and L-phenylalanyl-lactose; Hypertension

1. Introduction

Hypertension is a leading cause of health concern all over the world. Renin angiotensin system (RAS) plays a major role in the development of hypertension and angiotensin converting enzyme (ACE; EC.3.4.15.1) constitutes a key component in this system. ACE raises blood pressure by converting angiotensin-I, released from angiotensinogen by renin, into the potent vasoconstrictor angiotensin-II. ACE also degrades vasodilative bradykinin in blood vessels and stimulates the release of aldosterone in the adrenal cortex. Increased ACE activity has been linked to narrowing of lumen of blood vessels, which results in increased blood pressure [1]. Inhibition of ACE is considered to be an important therapeutic approach for controlling hypertension. ACE inhibitors (viz., captopril, enalapril, fosinopril and ramepril) currently available in the market, exert antihypertensive effect by competitively binding to the active site of ACE [2]. However, some of the currently used ACE inhibitors have certain limitations like susceptibility to proteolytic degradation leading to side effects such as brochospasm and dry cough [3]. As a result, there have been renewed efforts in modifying available drugs or in developing new drugs with lesser side effects.

During the past decades, several fundamental studies have led to the search for ACE inhibitors from natural resources. Certain functional foods containing ACE inhibitory compounds have been found to act as alternatives for treatment of hypertension. Most of the food-derived ACE inhibitors are peptidic in nature [4]. Only a few non-peptidic compounds have been reported to possess the ACE inhibitory activity [5]. Park et al. [6] recently discussed the effectiveness of chitooligosaccharides for ACE inhibition while Huang et al. [7] have reported improved ACE inhibitory activity of chitooligosaccharides by carboxyl modification. Interestingly, the existence of carbohydrate-binding center on ACE has been proposed recently [8,9]. While many carbohydrate-peptide conjugates are reported to display a wide variety of potent biological activities of therapeutic value [10–12], use of amino acid esters of carbohydrates as inhibitors of ACE has not been reported so far. The main objective of

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doi:10.1016/j.ijbiomac.2006.01.008

the present investigation was to enzymatically synthesize amino acid esters of carbohydrates and to determine their potential as ACE inhibitors in vitro.

2. Experimental

2.1. General methods

Rhizomucor miehei lipase (RML) immobilized on a weak anion resin was purchased from Nova Nordisk Denmark, porcine pancreas lipase (PPL) type II, steapsin and *Candida rugosa* lipase (CRL) were purchased from Sigma Chemical Co., USA. Esterification activities for RML, PPL and CRL were found be 0.46, 0.06 and 0.03 μmol/min/mg of enzyme, respectively [13]. L-Amino acids were procured from Hi-Media (India), D-glucose was purchased from SD fine chemicals (India) and lactose was obtained from SISCO Research laboratories (India). Sephadex G-25 was purchased from Pharmacia, Sweden. All solvents used were of analytical grade and were procured from Qualigens Fine Chemicals (India).

2.2. Synthesis of amino acid esters of carbohydrates

Esterification was carried out as reported earlier [14] in presence of RML in case of L-alanyl-D-glucose and L-phenylalanyl-D-glucose, CRL in case of L-valyl-D-glucose and PPL in case of L-phenylalanyl-lactose (40% enzyme based on w/w of D–glucose/lactose) by refluxing with stirring 0.005 mol of amino acid (L-alanine, L-valine, L-phenylalanine) and 0.005 mol of D-glucose or 0.0025 mol of lactose in a 100 mL solvent mixture consisting of CH_2Cl_2:DMF (90:10 v/v) or hexane:$CHCl_3$:DMF (45:45:10 v/v) in two necked flat bottom flask for 3 days at 40 °C (boiling point of the dichloromethane). The enzyme was imparted with 'pH memory' by employing: 0.1 mL Na_2HPO_4 buffer (0.1 mM, pH 7.0) for L-valyl-D-glucose, 0.1 mL CH_3COONa buffer (0.1 mM, pH 4.0) for L-alanyl-D-glucose, 0.2 mL CH_3COONa buffer (0.2 mM, pH 4.0) for L-phenylalanyl-D-glucose and L-phenylalanyl-lactose. To facilitate complete removal of water from the reaction mixture, a very low water activity of $a_w = 0.0054$ was achieved by condensing azeotropic solvent vapor containing small amount of water of reaction into a desiccant which was then drained back into the reaction mixture [14–17]. The same reactions were carried out in the absence of lipase, which did not show any esterification. After completion of the reaction, the solvent was distilled off and then stirred with 20 mL of water and filtered to remove the lipase. The filtrate containing unreacted substrates and product esters was evaporated to dryness on a water bath and analyzed by HPLC employing a C-18 column or an aminopropyl column [14,17]. Conversion yields were determined from HPLC peak areas of the ester and L-amino acid and expressed as percentage esterification with respect to the L-amino acid concentration. L-Alanyl-D-glucose and L-phenylalanyl-D-glucose were separated from the reaction mixture by repeated HPLC injections and L-valyl-D-glucose and L-phenylalanyl-lactose separated by size exclusion chromatography using Sephadex G-25 with water as eluant. All the esters were characterized by UV, IR and two-dimensional Heteronuclear Single Quantum Coherence Transfer (2-D-HSQCT) NMR spectra. Two-dimensional HSQCT-NMR spectra were recorded on a Bruker DRX-500 MHz spectrometer at 40 °C by dissolving 40 mg of the sample in DMSO-d_6.

2.3. ACE inhibitory assay

2.3.1. Preparation of ACE

ACE was prepared from porcine lung acetone powder by following the standard procedure [18]. Fresh pig lung was homogenized with 5–10 volumes of cold acetone in a waring blender. The homogenate was filtered and the residue was dried to remove the acetone. The dried lung acetone powder was stored at −20 °C and used for ACE extraction. ACE was extracted from the acetone powder as follows: the acetone powder was suspended in 10 volumes of Tris–HCl buffer (125 mM, pH 8.3) containing 1 M NaCl and homogenized. The homogenate was stirred overnight at 4 °C, and then centrifuged at 30,000 × *g*, for 20 min at 4 °C. The supernatant (the enzyme) was separated and was stored at −20 °C.

2.3.2. Determination of ACE activity

ACE activity was determined spectrophotometrically by a modification of the method of Cushman and Cheung [19] using Hippuryl-histidyl-leucine as the substrate. Twenty-five millimole HHL and appropriate quantity of ACE inhibitor were dissolved in Tris–HCl buffer (125 mM, pH 8.3) and incubated with the purified ACE for 60 min at 37 °C. The reaction was terminated by addition of 500 μl of 1 N HCl. The hippuric acid formed by the action of ACE was extracted into 1500 μl of ethyl acetate. After centrifugation at 800 × *g* for 10 min, 1000 μl of supernatant was transferred to a separate clean dry test tube and was evaporated to dryness at 80 °C. The residue was dissolved in 1.0 ml of distilled water and the absorbance was measured at 228 nm against distilled water. Concentration-dependent activities of inhibitors were studied and the concentration required to inhibit 50% of ACE activity was defined as the IC_{50} value.

3. Results and discussion

3.1. Chemistry

Two-dimensional heteronuclear single/multiple quantum coherence transfer (HMQCT/HSQCT) NMR data confirmed that three diasteriomeric mono esters (2-*O*-, 3-*O*- and 6-*O*-esters) and two diasteriomeric diesters (2,6-di-*O*- and 3,6-di-*O*-esters) were formed in various proportions in case of L-valyl and L-phenylalanyl esters of D-glucose and two diasteriomeric mono esters (6-*O*- and 6′-*O*-esters) and one diasteriomeric diester (6,6′-di-*O*-ester) were formed in case of L-phenylalanyl-lactose (Table 1). In case of L-alanyl-D-glucose only β-D-glucose was found to have reacted to give three mono and two di esters (Fig. 1). The composition of various esters were as follows: L-alanyl-D-glucose, mixture of five diastereomeric esters:-6-*O*-46.7%; 3-*O*-11.5%; 2-*O*-19.9%; 2,6-di-*O*-6.6% and 3,6-di-*O*-15.3% from the total yield of 26.5%; L-valyl-D-glucose, mixture of five diastereomeric

Table 1
Composition of mono and diesters of amino acyl-D-glucose esters and their average molecular weights[a]

Ester	6-*O*-Ester (%)	3-*O*-Ester (%)	2-*O*-Ester (%)	3,6-di-*O*-Ester (%)	2,6-di-*O*-Ester (%)	6'-*O*-Ester (%)	6,6′-di-*O*-Ester (%)	Average molecular weight
L-Alanyl-D-glucose[b]	46.7	11.5	19.9	15.3	6.6	–	–	266.5
L-Valyl-D-glucose[c]	32.4	26.5	26.4	8.8	5.9	–	–	293.5
L-Phenylalanyl–D-glucose[b]	24.1	23.3	19.2	16.8	16.6	–	–	376.3
L-Phenylalanyl-lactose[c]	42.1	–	–	–	–	30.9	27.0	528.7

[a] Percentage compositions of all five esters as determined from ^{13}C peaks of C-6 of esters.
[b] L-Alanyl-D-glucose and L-phenylalanyl-D-glucose were separated from the reaction mixture by repeated HPLC injections to obtain yields of 26.5% and 92.4%, respectively.
[c] L-Valyl-D-glucose and L-phenylalanyl-lactose were separated using sephadex G-25 to obtain yields of 68.2% and 50.58% respectively.

esters:-6-*O*-32.4%; 3-*O*-26.5%; 2-*O*-26.4%; 2,6-di-*O*-8.8% and 3,6-di-*O*-5.9% from the total yield of 68.2%; L-phenylalanyl-D-glucose mixture of five diastereomeric esters:-6-*O*-24.1%; 3-*O*-23.3%; 2-*O*-19.2%; 2,6-di-*O*-16.6% and 3,6-di-*O*-16.8% from the total yield of 92.4% and L-phenylalanyl-lactose mixture of three diastereomeric esters:-6-*O*-42.1%; 6'-*O*-30.9%; and 6,6'-di-*O*-27.0% from the total yield of 50.58%.

3.1.1. L-*Alanyl*-D-*glucose esters*

UV: 294.0 nm ($\log \varepsilon - 2.883$), 227.0 nm ($\log \varepsilon - 3.061$); IR: 3371 cm^{-1} (NH), 3410 cm^{-1} (OH), 2997 cm^{-1} (CH) and 1653 cm^{-1} (C=O); specific rotation-$[\alpha]_D$ at 25 °C = −38.14°; CMC–2.25 mM (0.056%).

2-*O*-Ester: ^1H NMR δ_{ppm}: (500.13 MHz): 2.95 (αCH), 1.30 (βCH$_3$), 3.62 (H-2β), 3.83 (H-3β), 3.67 (H-4β), 3.44 (H-6β); ^{13}C NMR δ_{ppm} (125 MHz): 52.1(αCH), 15.7(βCH$_3$), 100.2 (C$_1$β), 82.6 (C$_2$β), 77.9 (C$_3$β), 68.8 (C$_4$β), 60.5 (C$_6$β). 3-*O*-Ester: ^1H NMR δ_{ppm}: 2.87(αCH), 3.93 (H-3β), 3.58 (H-4β), 3.82(H-6β); ^{13}C NMR δ_{ppm}: 51.4 (αCH), 83.3 (C$_3$β), 69.3 (C$_4$β), 63.5 (C$_3$β). 6-*O*-Ester: ^1H NMR δ_{ppm} 2.95 (αCH), 1.30 (βCH$_3$), 3.86(H-2β), 3.4(H-4α), 3.76(H-5β), 3.36(H-6β); ^{13}C NMR δ_{ppm}: 50.2(αCH), 15.1(βCH$_3$), 171.4(CO), 101.8(C$_1$β), 65.4 (C$_6$β), 75.0 (C$_2$β), 70.1(C$_5$β), 57.3 (C$_6$β). 2,6-di-*O*-Ester: ^1H NMR δ_{ppm}: 3.36 (αCH), 1.30 (βCH$_3$), 3.78 (H-2β), 3.47 (H-6β), ^{13}C NMR δ_{ppm}: 49.5(αCH), 16.4 (βCH$_3$), 76.5 (C$_2$β), 62.7 (C$_6$β). 3,6-di-*O*-Ester: ^1H NMR δ_{ppm}: 1.30 (αCH), 3.78 (H-3β), 3.82 (H-6β) ^{13}C δ_{ppm}: 16.7 (αCH), 81.6 (C$_3$β), 63.1(C$_6$β).

3.1.2. L-*Valyl*-D-*glucose esters*

UV: 275.0 nm ($\log \varepsilon - 2.4$), 227.0 nm ($\log \varepsilon - 2.65$); IR: 3394 cm^{-1} (NH), 2985 cm^{-1} (OH), 2971 cm^{-1} (CH) and 1638 cm^{-1} (C=O); specific rotation-$[\alpha]_D$ at 25 °C = +30.0°.

2-*O*-Ester: ^1H NMR δ_{ppm} (500.13 MHz): 3.06 (αCH), 2.15 (βCH), 1.07 (γCH$_3$), 3.89 (H-2α), 3.75 (H-2β), 3.51 (H-6α); ^{13}C NMR δ_{ppm} (125 MHz): 53.4 (αCH), 20.9(βCH), 9.51 (γCH$_3$), 76.1 (C$_2$α), 60.0 (C$_6$α). 3-*O*-Ester: ^1H NMR δ_{ppm}: 3.10 (αCH), 0.94 (γCH$_3$), 3.89 (H-3α), 4.01 (H-3β), 3.33 (H-6αβ); ^{13}C NMR δ_{ppm}: 52.4 (αCH), 9.39 (γCH$_3$), 82.9 (C$_3$α), 83.4 (C$_3$β), 60.3 (C$_6$αβ). 6-*O*-Ester: ^1H NMR δ_{ppm}: 3.20 (αCH), 2.01 (βCH), 0.90 (γ CH$_3$), 4.95 (H-1α), 4.22 (H-1β), 3.17 (H-4α), 3.0 (H-4β), 3.86 (H-6α); ^{13}C NMR δ_{ppm}: 51.9 (αCH), 21.0 (βCH), 8.94 (γ CH$_3$), 95.2 (C$_1$α), 104.5(C$_1$ β), 69.5 (C$_4$α), 69.8 (C$_4$β), 63.4 (C$_6$α). 2,6-di-*O*-Ester: ^1H NMR δ_{ppm}: 3.15 (αCH), 3.75 (H-2α), 3.64 (H-6β), ^{13}C NMR δ_{ppm}: 51.7 (αCH), 78.7 (C$_2$α), 61.6 (C$_6$α). 3,6-di-*O*-Ester: ^1H NMR δ_{ppm}: 3.21 (αCH), 1.55 (γ CH$_3$), 3.67 (H-3β), 3.15 (H-6αβ); ^{13}C NMR δppm: 49. 4 (αCH), 78.6 (C$_3$β), 61.3 (C$_6$αβ).

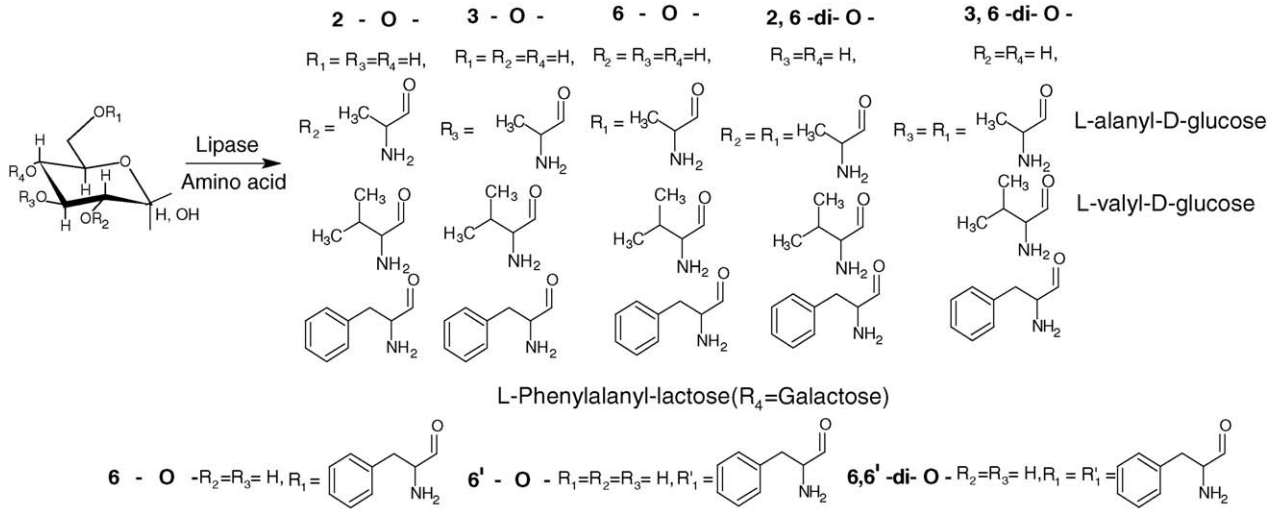

Fig. 1. Schematic representation for the lipase catalysed synthesis of L-amino acid esters of carbohydrates.

3.1.3. L-Phenylalanyl D-glucose esters

UV: 308.0 nm ($\log \varepsilon - 2.79$), 257.5 nm (3.08) and 237.0 nm ($\log \varepsilon - 3.12$); IR: 3642 cm^{-1} (NH), 3164 cm^{-1} (OH), 1722 cm^{-1} (C=O) and 1582 cm^{-1} (aromatic); specific rotation-$[\alpha]_D$ at 25 °C = −24.2; CMC–3.25 mM (0.106%).

2-*O*-Ester: ^{1}H NMR δ_{ppm} (500.13 MHz): 2.92(αCH), 2.51(βCH_{2a}), 4.6(H-1α), 3.67(H-2α), 3.4(H-6α); ^{13}C NMR δ_{ppm} (125 MHz): 52.0(αCH), 35.8(βCH_2), aromatic-136.5 (C_1), 96.3($C_1\alpha$), 77.1($C_2\alpha$), 62.0($C_6\alpha$). 3-*O*-Ester: 1H NMR δ_{ppm}: 3.01(αCH), 3.11(βCH_{2a}), 2.96(βCH_{2b}), 4.4 (H-1α), 3.61 (H-2α), 3.66(H-2β), 3.82(H-3α), 3.91(H-3β), 3.40(H-6α); ^{13}C NMR δ_{ppm}: 53.0(αCH), 36.8(βCH_2), aromatic-136.4 (C_1), 97.3($C_1\alpha$), 83.4($C_3\alpha$), 83.9($C_3\beta$), 61.9($C_6\alpha$). 6-*O*-Ester: ^{1}H NMR δ_{ppm}: 3.07(αCH), 3.18(βCH_{2a}, $J = 12.1$); 3.06(βCH_{2b}, $J = 12.1$), aromatic-7.18 (H2,6, $J = 3.5$), 7.26(H3,5, $J = 8.74$), 7.16(H4), 3.16(H-5α), 3.78(H-6α), 3.66(H-6β); ^{13}C NMR δ_{ppm}: 54.2(αCH), 36.7(βCH_2) aromatic-136.3(C_1),128.9(C_2, C_6), 130.7(C_3, C_5), 130.3(C_4), 172.5(CO), 102.2($C_1\alpha$), 70.5($C_5\alpha$), 65.0($C_6\alpha$, $C_6\beta$). 2,6-di-*O*-Ester: ^{1}H NMR δ_{ppm} 3.51(H-6α), 3.61(H-6β), 3.67(H-2α); ^{13}C NMR δ_{ppm} 77.0($C_2\alpha$), 79.0($C_2\beta$), 62.1($C_6\beta$). 3,6-di-*O*-Ester: ^{1}H NMR δ_{ppm} 3.50(H-6α), 3.61(H-3α), 3.66(H-3β); ^{13}C NMR δ_{ppm} 82.3($C_3\alpha$), 83.4($C_3\beta$), 64.8($C_6\alpha$).

3.1.4. L-Phenylalanyl-lactose

UV: 308.0 nm ($\log \varepsilon - 2.77$), 258.0 nm ($\log \varepsilon - 3.00$) and 237.5 nm ($\log \varepsilon - 3.48$); IR: 3550 cm^{-1} (NH), 3383 cm^{-1} (OH), 1701 cm^{-1} (C=O) and 1556 cm^{-1} (aromatic).

6-*O*-Ester: ^{1}H NMR δ_{ppm} (500.13 MHz): 2.67(αCH), 2.89(βCH_{2a}), aromatic-7.25 (H2,6), 7.28(H3,5), 7.2(H4), 4.32(H-1α), 4.23(H-1β), 3.36(H-2α), 3.49(H-2β), 3.32(H-3α), 3.80(H-3β), 3.56(H-4α), 3.72(H-4β), 3.90(H-5αβ), 3.47(H-6αβ), 4.16(H′-1β), 3.14(H′-2β), 3.42(H′-3β), 3.62(H′-4β), 3.39(H′-5β), 3.54(H′-6β); ^{13}C NMR δ_{ppm} (125 MHz): 51.9(αCH), 38.2(βCH_2) aromatic-138.0(C_1),128.3(C_2, C_6), 129.1(C_3, C_5), 126.1(C_4), 172.5(CO), 96.6($C_1\alpha$), 101.2($C_1\beta$), 70.5($C_2\alpha$), 73.0(C_2 β), 74.0($C_3\beta$), 82.5($C_4\alpha$), 83.3($C_4\beta$), 77.2($C_5\alpha\beta$), 62.1($C_6\alpha\beta$), 103.2($C_1'\beta$), 69.9 (C_2' β), 71.6($C_3'\beta$), 68.2($C_4'\beta$), 74.4($C_5'\beta$), 60.5($C_6'\beta$); 6′-*O*-Ester: ^{1}H NMR δ_{ppm}: 2.72(αCH), 3.00(βCH_{2a}), 5.16(H-1α), 4.32(H-1β), 3.16(H-2α), 3.45(H-2β), 3.43(H-3α), 3.50 (H-3β), 3.32(H-4α), 3.80(H-5αβ), 3.49(H-6αβ), 4.15(H'-1β), 3.36(H′-2β), 3.89(H′-4β), 3.89 (H′-5β), 3.45(H′-6β); ^{13}C NMR δ_{ppm}: 52.5(αCH), 37.1(βCH_2) aromatic-137.6(C_1), 92.0($C_1\alpha$), 97.1($C_1\beta$), 69.7($C_2\alpha$), 72.7($C_2\beta$), 72.1($C_3\alpha$), 74.5($C_3\beta$), 79.9($C_4\alpha$), 80.7($C_4\beta$), 76.0($C_5\alpha\beta$), 61.0($C_6\alpha\beta$), 104.0(C_1' β), 69.3 (C_2' β), 72.1(C_3' β), 66.3(C_4' β), 75.0(C_5' β), 63.1($C_6\beta$); 6,6′-di-*O*-Ester: ^{1}H NMR δ_{ppm}: 2.55(αCH), 2.89(βCH_{2a}), 4.32(H-1α), 3.23(H-2α), 3.68(H-2β), 3.33(H-3α), 4.02(H-4α), 4.01(H-5αβ), 3.45(H-6αβ), 4.21(H′-1β), 3.14(H′-2β), 3.58(H′-3β), 3.74(H′-4β), 3.45(H′-6β); ^{13}C NMR δ_{ppm}: 51.7(αCH), 37.9(βCH_2) aromatic-137.8(C_1), 95.6($C_1\alpha$), 101.9($C_1\beta$), 71.3($C_2\alpha$), 73.2(C_2 β), 73.3($C_3\alpha$), 74.7($C_3\beta$), 82.2($C_4\alpha$), 84.2($C_4\beta$), 77.7($C_5\alpha\beta$), 62.4($C_6\alpha\beta$), 103.4(C_1' β), 69.9 (C_2' β), 72.4(C_3' β), 67.4(C_4' β), 74.7(C_5' β), 62.7($C_6\beta$).

Riva et al. [20] reported that three different mono esters of *N*-acetyl L-phenylalanyl-D-glucose were formed when reaction was carried out in presence of subtilisin (2-*O*-, 3-*O*- and 6-*O*-mono esters). Tamura et al., [21] have reported synthesis of 2-*O*-and 3-*O*-mono esters of L-valyl-D-glucose chemically. Park et al. [22] have reported formation of three different esters of *N*-acetyl L-phenylalanyl-lactose (6-*O*-, 6′-*O*- and 6,6′-di-*O*-esters). The present work reports the synthesis of the 18 esters (mono and diesters) of which the following 10 esters have not been reported so far: 2-*O*-L-alanyl-D-glucose, 3-*O*-L-alanyl-D-glucose, 6-*O*-L-alanyl-D-glucose, 2,6-di-*O*-L-alanyl-D-glucose, 3,6-di-*O*-L-alanyl-D-glucose, 6-*O*-L-valyl-D-glucose, 2,6-di-*O*-L-valyl-D-glucose, 3,6-di-*O*-L-valyl-D-glucose, 2,6-di-*O*-L-phenylalanyl-D-glucose and 3,6-di-*O*-L-phenylalanyl-D-glucose. The average molecular weight of the esters in case of L-alanyl and L-phenylalanyl-D-glucose was evaluated from the percentage composition of all five esters determined from the C-6 peak area from ^{13}C spectra.

Table 2
ACE inhibitory activities of the amino acid esters of carbohydrates

Ester	ACE inhibition IC_{50}, mM[a]
L-Alanyl-D-Glucose	0.230 ± 0.02
L-Valyl-D-Glucose	0.396 ± 0.03
L-Phenylalanyl-D-Glucose	0.121 ± 0.01
L-Phenylalanyl-lactose	0.229 ± 0.01

[a] Mean ± S.E. of three independent determinations. Respective L-alanine, L-valine, L-phenylalanine, D-glucose and lactose as controls showed no ACE inhibitory activity when tested at 0.2 mM.

3.2. Biological evaluation

The synthesized amino acid esters of carbohydrates were evaluated for their ability to inhibit angiotensin-converting enzyme (ACE). All the amino acid esters (in the form of mixtures) showed moderate potency as inhibitors of ACE (Table 2, Fig. 2). While L-phenylalanyl-D-glucose showed highest ACE inhibitory activity with an IC_{50} of 0.121 mM, L-valyl-D-glucose showed lowest ACE inhibitory activity with an IC_{50} of 0.396 mM. The other two esters showed intermediate IC_{50} value of 0.23 mM. Although D-glucose esters of L-alanine

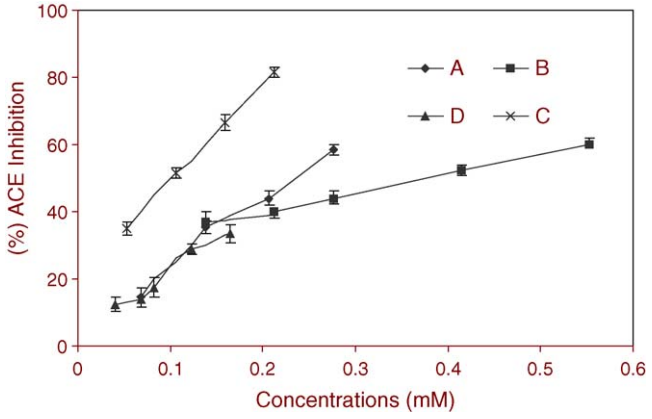

Fig. 2. ACE inhibitory patterns of L-amino acid esters of carbohydrates. Each value is a Mean ± S.E. of three determinations. ♦: L-alanyl-D-glucose; ■: L-valyl-D-glucose; ×: L-phenylalanyl-D-glucose; ▲: L-phenylalanyl-lactose.

and L-valine exhibited good in vitro inhibitory activity against ACE, L-phenylalanine esters were the most effective. Recent studies [9] have shown that monosaccharides contribute an important role in forming the supramolecular structure of ACE and also inhibit ACE dimerisation. There are reports that dipeptides containing aromatic amino acids – phenylalanyl-leucine, tryptophanyl-leucine, tyrosinyl-leucine and tryptophanyl-proline – exhibited relatively high inhibitory activity against ACE [23,24]. Non peptide prodrugs such as amino acid conjugates of acyclovir, cephalexin and bestatin were reported to be absorbed by H^+-coupled peptide transporters even though they contain highly polar groups [25]. All these dipeptides and non peptide prodrugs contain free amino groups (probably charged) attached to the α-CH of the amino acids with an adjacent –CO–NH– peptide linkage and aromatic or alkyl side chains which could be the probable binding groups from the drugs to the active site resulting in inhibition. The same functional portion is also present in the amino acid esters of carbohydrates reported herein, the only difference being the presence of –CO–O– linkage in the esters prepared instead of –CO–NH– from the reported drugs. This could be the reason for ACE inhibition by the esters reported in this work. Further, the amino acid residues are proposed [26] to interact through their hydrophobic side chains and block the ACE active site, thereby bestowing ACE inhibition. However, in our studies, free amino acids, D-glucose or lactose when tested at 0.2 mM did not show ACE inhibitory activity. Hence the inhibition obtained in the present study was probably due to a mixture of either all or some of the five esters in L-alanyl-D-glucose, L-valyl-D-glucose and L-phenylalanyl-D-glucose and three esters in L-phenylalanyl-lactose.

This implies that anchoring an amino group on a potent active site binding molecule like D-glucose/lactose possessing hydroxyl groups, capable of hydrogen bonding and hydrophobic interactions with some of the active site of the enzyme is essential. Since the esters in the mixtures possessed the same polarity, it was difficult to isolate the esters individually by chromatographic or other means. But, the esters were separated from the respective unreacted amino acids and D-glucose. However, potency of individual esters was not determined. But we speculate that some of the individual mono- and di-esters might possess more ACE inhibitory activity than the others.

4. Conclusions

The results from the present study indicate a potential role for the amino acid esters of carbohydrates as inhibitors of ACE. The structural approach of ACE inhibitors developed in the present work can be used with further suitable modifications in the carbohydrate moiety with the insertion of different combinations of amino acids to obtain more potent ACE inhibitors.

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