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Complex of Dipeptidyl Peptidase II with Adenosine Deaminase

S. G. Sharoyan[1], A. A. Antonyan[1], S. S. Mardanyan[1]*, G. Lupidi[2], M. Cuccioloni[2], M. Angeletti[2], and G. Cristalli[3]

[1]Buniatyan Institute of Biochemistry, Armenian National Academy of Sciences, 5/1 P. Sevak Str., Yerevan 0014, Armenia; fax: +374 (10) 29-73-43; E-mail: biochem@ipia.sci.am
[2]Department of Biology M. C. A. Sciences, University of Camerino, 2 Via Camerini, 62032 Camerino (MC), Italy; fax: +39 (0737) 403290; E-mail: giulio.lupidi@unicam.it
[3]Department of Chemical Sciences, University of Camerino, 1 Via S. Agostino, 62032 Camerino (MC), Italy; fax: 39 (0737) 402252; E-mail: gloria.cristalli@unicam.it

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Abstract—Dipeptidyl peptidase II (DPPII) from bovine kidney cortex and lung was purified to the electrophoretically homogeneous state. The molecular and catalytic characteristics of the enzyme were determined. It was revealed that DPPII preparations possess adenosine deaminase (ADA) activity at all purification steps. For the first time, the ADA-binding ability of DPPII has been shown similar to the well-known ADA-binding enzyme, DPPIV. The dissociation constant of the DPPII–ADA complex was estimated using a resonant mirror biosensor (80 nM), fluorescence polarization (60 nM), and differential spectroscopy (36 nM) techniques. The data demonstrate that DPPII can form a complex with ADA, but with one order of magnitude higher dissociation constant than that of DPPIV (7.8 nM).

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Dipeptidyl peptidases are unique serine proteases that remove a dipeptide from N-terminus of polypeptides and proteins containing preferentially proline or alanine in the penultimate position. This protease family includes a number of peptidases that are similar in their catalytic triad, but differ in substrate and inhibitor specificity, pH optimum, and localization of the scissile bond [1-3]. Among the natural substrates of these peptidases are chemokines, neuropeptides, hormones, growth factors, and other regulatory peptides the biological activity of which might be modulated under the action of these enzymes [4-6].

Dipeptidyl peptidase II (DPPII; EC 3.4.14.2) was first identified in bovine pituitary extract [7] and then in different tissues of various species, mainly in soluble form [4, 8, 9]. Researchers suggest that DPPII participates in degradation of collagen fragments, myofibril proteins, and neuropeptides, in cell differentiation, pathogenesis of autoimmune diseases, and protection from cell death [10-12]. The changes in the level and distribution of the enzyme indicate its possible role in disease-related processes. Thus, the influence of neurodegenerative processes on the level of DPPII activity, its increase in different brain regions with destroyed neurons, and the participation of the enzyme in cicatrization after penetrating injuries of the brain were proposed [13].

Another proline specific peptidase, dipeptidyl peptidase IV (DPPIV; EC 3.4.14.5), known also as activated T-cells antigen CD26, is the most intensively studied member of the dipeptidase family. It is a ubiquitous, multifunctional integral type II glycoprotein located on the surface of epithelial, endothelial, and lymphoid cells [14, 15]. Rather recently, the identity of DPPIV [16] and the well-known adenosine deaminase-binding protein without any known activity [17] has been proved. Adenosine deaminase (ADA; EC 3.5.4.4), a key enzyme in purine metabolism catalyzing (deoxy)adenosine deamination to (deoxy)inosine [18], is widely distributed in mammalian tissues and participates in the development and function of lymphoid cells. ADA binds specifically to human, bovine, and rabbit (but not rodent) DPPIV with dissocia-

Abbreviations: ADA) adenosine deaminase; DPP) dipeptidyl peptidase; Fluo-ADA) fluorescein isothiocyanate-labeled ADA; Gly-Pro-pNA) Gly-Pro-*p*-nitroanilide.

* To whom correspondence should be addressed.

tion constant of 3-20 nM depending on the species [14]. DPPIV is known as the only dipeptidyl peptidase with the ability to bind ADA. Other enzymes of the dipeptidyl peptidase family—FAPα/seprase, DPP8, DPP9, etc.—homologous with DPPIV, do not bind ADA [5].

In spite of the similarity in substrate (natural and synthesized) specificity and catalyzed enzymatic reaction, DPPIV and DPPII differ in molecular mass, pH optimum of catalytic activity, cellular localization, and, probably in physiological role. In the present report, data indicating the ability of DPPII to form a complex with ADA, similar to DPPIV, are described for the first time. The dissociation constant of the DPPII–ADA complex was evaluated using resonant mirror biosensor, fluorescence polarization, and differential spectroscopy methods. Based on these observations we suggest that like DPPIV, DPPII has some unknown biological (physiological) activities that might involve interaction with ADA.

MATERIALS AND METHODS

Materials. Adenosine, Gly-Pro-pNA, ovalbumin, BSA, fluorescein isothiocyanate, and intestinal ADA (type VI) were purchased from Sigma (USA), DEAE-cellulose was from Whatman (England), and Sephadex G-25 and G-200 and DEAE-Sephadex A-50 were from Pharmacia Biotech (Sweden). ADA from bovine lung, cytochrome *c* from bovine heart, and γ-globulin from human plasma were isolated and purified to electrophoretic homogeneity in our laboratory. Other reagents were of the highest available purity.

Fluorescence was measured in a Shimadzu RF 5301PC spectrofluorimeter (Japan) fitted with a polarizer, and spectral and kinetic measurements were performed in Specord M-40 (Germany) and Cary 1 (Varian, USA) UV-VIS spectrophotometers. The measurements were performed at 25°C using thermostatted cuvette holders. HPLC was performed in a Superose 6 column on an Alltech HPLC equipped with a UV-VIS recorder.

DPPII was isolated and purified from bovine kidney cortex and lung. The main purification procedures were performed at 4°C. After removing the cover, 150-200 g of tissue was scissored, washed in 0.9% NaCl, and homogenized for 1.5 min in phosphate buffer containing 8 mM Na_2HPO_4/2 mM KH_2PO_4, pH 7.4 (buffer A), at a ratio of 1 : 5 (w/v). After centrifugation of the homogenate for 20 min at 15,000 rpm, DEAE-cellulose suspension equilibrated in buffer A was added to the supernatant at a ratio 1 : 10 (v/v). The mixture was stirred on magnetic stirrer for 45 min. After settling, the cellulose was transferred into a glass column and sequentially washed with buffer A without and with 20 mM KCl. The proteins adsorbed on the cellulose were eluted with buffer A containing 0.3 M KCl. The eluted fractions, possessing DPP activity, were subjected to gel filtration on Sephadex G-200 equilibrated with buffer A containing 0.1 M KCl (buffer B) at flow rate of 0.5 ml/min. The fractions possessing DPPII activity (DPP activity at pH 5.5) were collected, dialyzed overnight against ten-fold volume of buffer A, and loaded on a column of DEAE-Sephadex A-50 equilibrated in buffer A. After washing the column with the same buffer, the proteins were eluted with a step gradient between 0.05-0.35 M KCl in buffer A. The fractions with DPPII activity were combined and exposed to acidic treatment: 0.2 M acetic buffer, pH 5.5, was added to 80 mM followed by incubation for 40 min at 37°C. The formed precipitate was removed by centrifugation. The supernatant was dialyzed against buffer A, concentrated on a Centriplus YM-10 centrifugal micro-concentrator (Millipore, USA), and subjected to gel filtration through a Sephadex G-200 column at flow rate of 0.2 ml/min. DPPII, DPPIV, and ADA activities were measured at all purification stages.

DPPII and DPPIV activities were assayed in identical procedures using different buffers: acetic buffer (pH 5.5) for DPPII assay and phosphate buffer (pH 7.5) for DPPIV. Usually 500 μl of assay mixture contained 40 mM buffer and an enzyme sample (5-10 μg protein). The reaction was initiated by addition of a stock solution of substrate in water (2 mM Gly-Pro-pNA) to final concentration of 0.24 mM. After incubation for a selected time at 37°C, the reaction was stopped by addition of acetic buffer, pH 4.2, final concentration 0.1 M. Turbidity was removed by centrifugation, and the amount of *p*-nitroaniline was evaluated from the absorption at 390 nm registered against the identically treated reference sample without the enzyme. The extinction coefficient of *p*-nitroaniline at 390 nm is 9.9 $mM^{-1} \cdot cm^{-1}$ [4].

The ADA activity was assayed by determination of ammonia liberated in the reaction of adenosine deamination using a phenol–hypochlorite colorimetric method described elsewhere [19].

The molecular mass of DPPII was estimated by gel filtration and electrophoresis. The electrophoresis was carried out in accordance with the method of Weber and Osborn [20] in 9% polyacrylamide gel in the presence of 0.1% SDS, 0.1% β-mercaptoethanol, and 6 M urea. As standards, γ-globulin (150 kD), BSA (67 kD), ovalbumin (44 kD), and cytochrome *c* (13 kD) were used. The gels were stained with Coomassie Blue G-250.

Protein was determined by the method of Bradford [21] (BSA was used as a standard) and/or spectrophotometrically from the difference in absorptions of the protein solution at 215 and 225 nm [22].

The pH dependence of DPPII activity was analyzed in the pH range 4.0-8.5 in increments of 0.5. Buffer containing 50 mM CH_3COONa, 50 mM KH_2PO_4, and 50 mM $Na_2B_4O_7$ was used. The desired pH was achieved by titrating with NaOH or HCl.

The interaction of DPPII with ADA was investigated using several techniques. Using the *gel-filtration* method, the mixture of proteins in buffer A was incubated at 25°C

for 2 h, stored overnight at 4°C, and subjected to gel filtration through a Sephadex G-200 column equilibrated in buffer B at flow rate of 0.2 ml/min. The specific activities of both enzymes were determined in the eluted fractions.

Using the *resonant mirror biosensor technique*, DPPII (or DPPIV) was immobilized on one of the cells of a carboxymethyl-dextran cuvette of an IAsys plus device (Affinity Sensors, UK) as described by Davies et al. [23]. The cuvette was rinsed with Tween-20-containing phosphate buffer (10 mM NaH_2PO_4, 2.7 mM KCl, 138 mM NaCl, 0.05% Tween-20, pH 7.4), equilibrated with the same buffer without Tween-20 to establish a base line, and the enzyme was covalently immobilized onto the cuvette matrix by the standard 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/N-hydroxysuccinimide coupling procedure. The non-coupled ligand was removed by washing with the buffer, and the remaining reactive groups were deactivated by sequential washing with 1 M ethanolamine (pH 8.5), 10 mM HCl, and buffer. The amount of immobilized DPP was calculated based on the display of the device, and ADA was added at definite concentrations. Binding experiments were performed at 25°C, and the data were analyzed using the "Fast Fit" computer program (Fison Applied Sensor Technology) supplied with the instrument.

For the *fluorescence polarization technique*, intestinal ADA labeled with fluorescein isothiocyanate was used. For this, the protein was incubated for 2 h at 37°C in 0.1 M phosphate buffer, pH 7.4, containing 30 μM fluorescein isothiocyanate. The mixture was subjected to gel filtration through Sephadex G-25 to remove the excess dye. The resulting Fluo-ADA was incubated with different concentrations of DPPII, and the fluorescence of ADA-bound fluorescein was registered in both the horizontal (I_H) and vertical (I_V) orientations. The polarization of the emission (P) in DPPII–ADA complexes at different concentrations of DPPII and constant concentration of Fluo-ADA was calculated according to the polarization equation [24]:

$$P = (I_V - I_H)/(I_V + 2I_H).$$

The measurements were performed at excitation and emission wavelengths of 492 and 520 nm, respectively, and the slit width of both beams was 5 nm. The dissociation constant for the DPPII–ADA complex, K_d, was evaluated from the dependence of P on DPPII concentration by nonlinear regression analysis of the Scatchard plot. The interaction of Fluo-ADA with DPPII was studied at pH 6.0, near the optimum of DPPII activity and stability of ADA.

Using the *differential spectrophotometry* to obtain the differential spectra for DPPII and ADA binding, ADA and DPPII solutions in 80 mM phosphate buffer, pH 6.3, were filled into separate compartments of two tandem cuvettes with 0.2-cm light path of each section. After registering background zero (Specord spectrophotometer, AutoZero mode), the solutions in the two compartments of the sample cuvette were mixed, incubated for 15 min at 30°C, and the differential spectrum of the protein mixture against individual protein solutions in the separate compartments was registered. In the resulting differential spectra the increase in absorption at 252 nm was observed in correlation with the increase in the [ADA]/[DPPII] ratio. The dissociation constant of the DPPII–ADA complex, K_d, was evaluated by nonlinear regression analysis of the Scatchard plot for dependence of absorption intensity at 252 nm on ADA concentration.

Kinetic and statistical analyses were performed using GraFit [25] and InStat (version 3 for Windows) software.

RESULTS AND DISCUSSION

Isolation and purification of DPPII from bovine lung and kidney cortex. The primary separation of DPPII and DPPIV was based on the difference in their molecular masses. The fractions eluted from DEAE-cellulose possessing DPP activity were subjected to gel filtration through a Sephadex G-200 column. In Fig. 1, the elution profiles for the enzymes from kidney cortex are shown. An identical pattern was found for the preparation from lung.

Figure 1 (curve *3*) for DPP activity determined at pH 7.5 shows two peaks. Obviously, DPPIV is responsible for the high molecular weight peak of activity. Its molecular mass was evaluated based on the elution volume of this peak and was 240 kD for the enzyme from kidney cortex and 300 kD for the sample from lung. A minor low molecular weight peak of curve *3* coincides with the single peak of DPP activity determined at pH 5.5 (curve *4*) and might be considered as the activity of DPPII. The molecular mass of DPPII from kidney cortex and lung was evaluated as 150 and 200 kD, respectively.

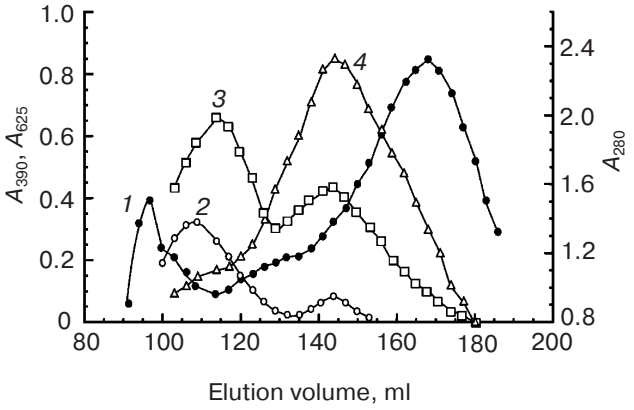

Fig. 1. Profiles of gel filtration through a Sephadex G-200 column of DEAE-cellulose eluate from kidney: *1*) protein concentration, A_{280}; *2*) ADA activity, A_{625}; *3*) DPP activity at pH 7.4, A_{390}; *4*) DPP activity at pH 5.5, A_{390}. Column size, 3 × 42 cm; void volume, 98 ml. Identical profiles were obtained for ADA preparations from lung.

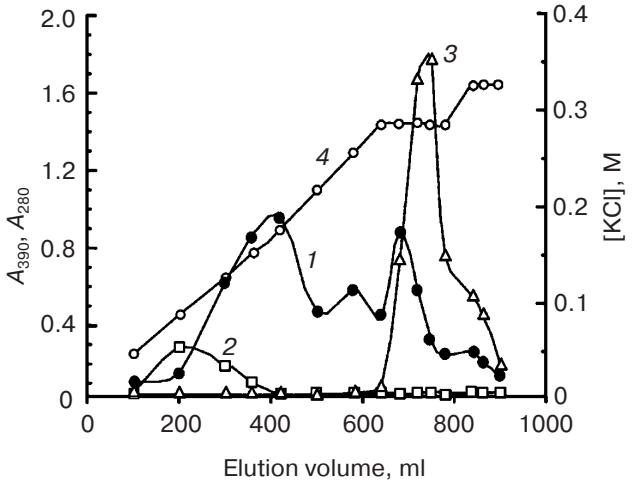

Fig. 2. Profiles from gradient elution of proteins from a DEAE-Sephadex A-50 column: *1*) protein concentration, A_{280}; *2*) DPP activity at pH 7.4, A_{390}; *3*) DPP activity at pH 5.5, A_{390}; *4*) concentration of KCl.

In the next purification step using ion-exchange chromatography on DEAE-Sephadex A-50, DPPII was completely separated from DPPIV owing to the difference in their isoelectric points (6.2 for DPPIV and 4.8 for DPPII [2]). The active fractions of DPPIV were eluted between KCl concentrations 0.1-0.15 M, while those of DPPII were eluted at 0.25 M and higher (Fig. 2). Then, to remove some residual weakly acidic contaminating proteins, the DPPII fraction was exposed to acidic treatment at pH 5.5. Being strongly acidic, the enzyme was not lost under this treatment.

During purification, it was noted that DPPII fractions possessed ADA activity at all steps, even after the last step of gel chromatography on Sephadex G-200. Monitoring the purity of the final preparations by HPLC proved the absence of any other protein peak besides that of DPPII.

The final preparations of DPPII from bovine kidney and lung exhibited one major band on SDS-PAGE corresponding to molecular masses of 54 and 60 kD, respectively, with a minor band near 35-40 kD (Fig. 3). The latter can be assigned to ADA dissociated from DPPII under denaturing conditions. This minor band is displayed clearly for the sample from lung but not from kidney. This observation might be explained by the high level of low molecular weight ADA in lung and its very low level in kidney cortex: for many gel filtrations we observed high ADA activity in the region of 35-40 kD in the case of lung extracts and only traces for the extracts from kidney. This observation is in accordance with a publication concerning the enzyme from human kidney [26].

Comparing the SDS-PAGE and gel-filtration data and taking into account the ability of DPPII to form a complex with ADA, we suggest that:

– DPPII is a 110-120 kD homodimer of 54-60 kD subunits, depending on the tissue;

– DPPII from the kidney cortex with molecular mass 150 kD represents a complex of homodimer (110 kD) and one molecule of ADA (40 kD);

– DPPII from lung with molecular mass 200 kD represents a complex of a homodimer (120 kD) and two molecules of ADA (80 kD).

It is noteworthy that the porcine seminal plasma DPPII of molecular mass 200 kD was interpreted by Huang et al. [27] as being a result of trimer formation of identical 61-kD subunits. They did not consider the possibility of complex formation between a dimer of DPPII and ADA.

Some molecular and catalytic characteristics of the DPPII preparations from the two tissues are reported in Table 1. These data are in good agreement with the literature.

Ability of DPPII to complex with ADA. There is no available data concerning the ability of any DPP besides DPPIV to associate with ADA. Our experiments have shown that DPPII fractions, like those of DPPIV, possess ADA activity at all purification stages. The ADA activity of DPPII in the preparations from lung was higher than that

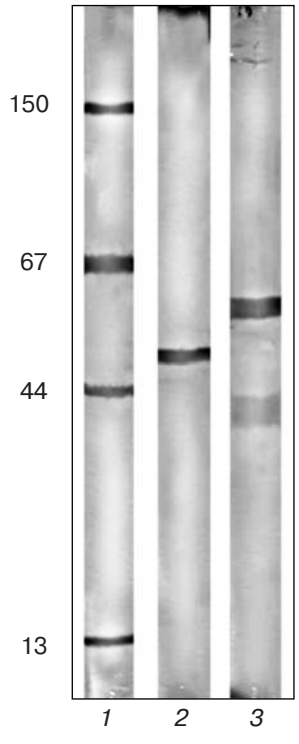

Fig. 3. SDS-PAGE of DPPII purified from bovine lung and kidney. After 9% PAGE, the gels were stained with Coomassie Brilliant Blue G-250. Lanes: *1*) standard proteins (γ-globulin, 150 kD; BSA, 67 kD; ovalbumin, 44 kD; cytochrome *c*, 13 kD); *2*) 12 μg of enzyme purified from bovine kidney; *3*) 20 μg of enzyme purified from lung.

Table 1. Molecular and catalytic characteristics of DPPII from bovine kidney and lung

Tissue	K_m, μM	k_{cat}*, sec^{-1}	pH optimum	Molecular mass, kD	
				gel filtration	SDS-PAGE
Kidney	340-360	10.8-11.5	5.5	140-150	51-54
Lung	1050-1100	6.5-6.8	5.5	180-200	60-62

* k_{cat} values were calculated based on the molecular mass of homodimer.

Table 2. Ratio of ADA activity to DPPII and DPPIV activities*

Purification step	ADA/DPPII		ADA/DPPIV	
	kidney	lung	kidney	lung
First gel filtration, G-200	0.1	59	13.6	63
Final gel filtration, G-200	0.03	14.5	7.2	20
Gel filtration after urea treatment, G-200	0.013	2.5	0.53	3.4

* All activities are determined for the same volume of the preparation under study.

from the kidney cortex, which is a consequence of the high level of free low molecular weight ADA in the lung. In Table 2, the ratios of ADA activity to DPPII and DPPIV activities at the three purification steps are presented. The data show that the purification decreased these ratios for both enzymes, indicating the separation of some ADA from them. However, up to the final purification step ADA activity was not completely removed from the DPP preparations. Moreover, we failed to remove ADA from peptidase preparations even by urea treatment. The purified preparation of DPPII was incubated for 30 min in buffer A containing 6 M urea and subjected to gel filtration through a Sephadex G-200 column equilibrated with buffer B, as was previously described for DPPIV [28]. The bottom row in Table 2 demonstrates the smaller but non-zero ADA/DPP activity ratios for both peptidases. It is interesting that the ADA/DPP activity ratios for two dipeptidases are similar in lung, whereas in kidney the activity ratio for DPPIV is two orders of magnitude higher than for DPPII. The ADA-binding sites in both enzymes in lung are probably saturated, but in the kidney cortex poor in free low molecular weight form, the enzymes compete and bind it in accordance with their affinity (see below).

Investigation of the ability of DPPII to bind ADA by gel filtration. A mixture of DPPII and ADA was incubated and subjected to gel filtration through a Sephadex G-200 column. In the control experiment, the identical preparation of DPPII without ADA was chromatographed. The comparison of protein elution profiles and ADA activities in DPPII fractions obtained in the presence and absence of ADA demonstrated a high molecular weight shift of the DPPII peak and 10-fold higher specific ADA activity in the former compared to the control. This suggests increasing amounts of ADA bound to DPPII.

Investigation of the ability of DPPII to bind ADA using the biosensor technique. Both DPPs from kidney were immobilized on the biosensor cuvette walls. Partial monolayers of proteins were formed; readout values for DPPII and DPPIV were 800 and 1800 arcsec, respectively. The ability of intestinal ADA to adsorb on the immobilized DPPII and DPPIV was studied by titration with increasing concentration of ADA. After reading the result of the interaction, the regeneration step was repeated up until baseline was achieved. The values of association/dissociation constants (k_{ass}/k_{diss}) were determined at two pH values for DPPII (6.2 and 7.4) and at pH 7.4 for DPPIV. In Table 3, the parameters for the studied interactions are presented as the means of at least three independent experiments. These parameters are strongly pH-dependent: the value of K_d for DPPIV near its activity optimum, pH 7.4, was 7.8 nM. It was very difficult to calculate this parameter at pH 6.2 because of very low affinity of DPPIV to ADA. The values of K_d for DPPII were 4.4 μM and 80 nM, respectively, at pH 7.4, and near the pH optimum of its enzymatic activity, 6.2. These values demonstrate, first, the highest affinity of each DPP for ADA at pH near of its own enzymatic activity optimum. Second, the affinity of ADA to DPPII is lower by an order of magnitude than the affinity to DPPIV. The kinetic analysis of the ADA binding to DPPII and DPPIV also demonstrated different recognition behavior: at pH 7.4, k_{ass} for DPPIV and ADA interaction is almost two orders of mag-

Table 3. Kinetic parameters for the interaction of intestinal ADA with DPPII and DPPIV from kidney determined by the biosensor technique

Enzyme	k_{ass}, $M^{-1}\cdot sec^{-1}$		$k_{diss} \times 10^{-3}$, sec^{-1}		K_d (k_{diss}/k_{ass}), μM	
	pH 7.4	pH 6.2	pH 7.4	pH 6.2	pH 7.4	pH 6.2
DPPII	1438 ± 102	5138 ± 131	6.3 ± 0.4	0.42 ± 0.24	4.4	0.0817
DPPIV	295749 ± 20702	n.d.	2.3 ± 0.2	n.d.	0.0078	n.d.

Note: n.d., not determined.

nitude higher than for DPPII and ADA, while k_{diss} at the same pH is only slightly affected.

Study of ADA binding to DPPII using fluorescence polarization. Fluo-ADA was titrated with increasing concentrations of DPPII from kidney, and the polarization of dye fluorescence was determined at each concentration. The formation of a complex between Fluo-ADA and the heavy molecule of DPPII resulted in significant polarization of the fluorescein emission. The value of K_d for DPPII–ADA complex was evaluated from the dependence of polarization on the concentration of DPPII (0.025-0.8 μM) at constant concentration of Fluo-ADA (0.8 μM). Nonlinear regression analysis of Scatchard plots for these data (Fig. 4) yielded K_d value of 60 ± 10 nM, which is close to the value of 80 nM obtained by the biosensor technique.

Study of ADA binding to DPPII using differential spectroscopy. Differential spectra of DPPII and ADA mixture were registered at increasing concentration of ADA. In Fig. 5, the differential spectrum with a maximum at 252 nm for the mixture of 0.8 μM DPPII from kidney and 0.4 μM ADA from lung in 80 mM K,Na-phosphate buffer, pH 6.3, is presented. DPPII was titrated with increasing concentrations of ADA up to [ADA]/[DPPII] ratios 0.1, 0.3, 0.5, 1.0, 1.5, and 2.0. The dissociation constant of the DPPII–ADA complex, K_d, evaluated by nonlinear regression analysis of the Scatchard plot, was 36 ± 5.6 nM. Inset, Scatchard transformation of the dependence of intensity at 252 nm against concentration of ADA. The K_d value obtained is lower than those obtained by the biosensor and fluorescence polarization techniques, but is of the same order of magnitude.

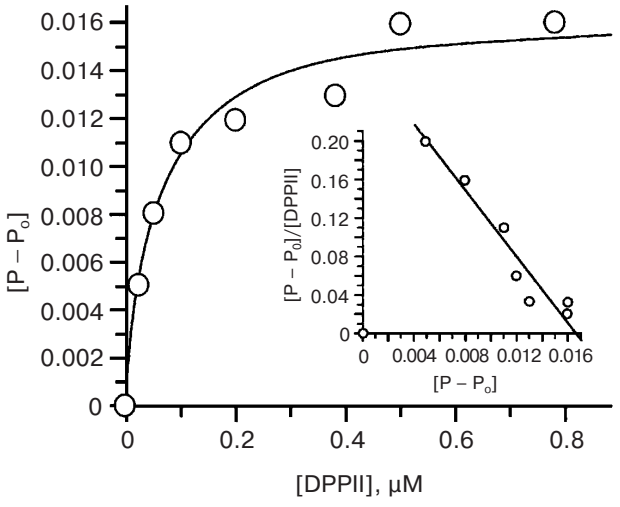

Fig. 4. Dependence of Fluo-ADA polarization on DPPII concentration. Fluo-ADA (0.8 μM) in 50 mM K,Na-phosphate buffer (pH 6.0) was titrated with increasing concentrations of DPPII from kidney. Inset, Scatchard plot for the interaction of DPPII with Fluo-ADA.

To investigate the physiological role of the less studied DPPII and to compare its molecular and catalytic characteristics with those of widely studied DPPIV, we isolated these two enzymes from bovine lung and kidney cortex.

Observation of ADA activity at all purification stages in DPPII preparations from both tissues suggested its ability to bind ADA, like DPPIV, with probable physiological consequences. To examine this suggestion, DPPII from kidney cortex seemed preferable because after purification it is less saturated with ADA (see Table 2). Our investigations with different techniques clearly showed the ability of bovine kidney cortex DPPII to bind ADA, although with affinity lower by one order of magnitude than that of DPPIV.

The study of crystal structure of DPPIV–ADA complex showed that the ADA-binding site is located on the β-propeller domain of DPPIV [29]. The amino acids in both ADA [30] and DPPIV [31] responsible for their interaction were identified. However, the physiological significance of complex formation between these enzymes is not yet clear.

Concerning the newly observed interaction between DPPII and ADA, we suggest that because DPPII does not have amino acid sequence homology with DPPIV [5], the ADA binding site on DPPII probably differs from that on DPPIV. The question about its structure will remain open until the crystal structure of DPPII is solved.

It is known that DPPII is localized in lysosomes and lysosome membranes, and ADA is localized both on the surface and in the cytoplasm of cells; therefore, their interaction cannot be excluded. It is interesting that the DPPII–ADA complex described in this work is similar to

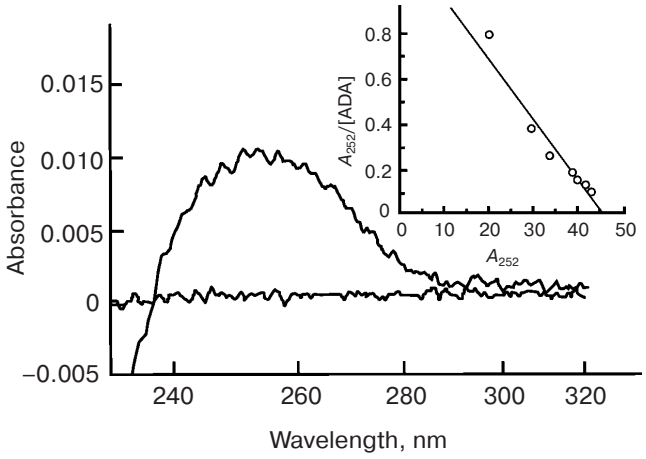

Fig. 5. Differential spectrum of DPPII–ADA complex. The zero line was registered for solutions of DPPII from kidney (0.8 μM) and ADA from lung (0.4 μM) in 80 mM K,Na-phosphate buffer, pH 6.3, in different compartments of tandem cuvettes in both the working and reference beams of the spectrophotometer. The differential spectrum is registered as absorption of mixture of the same solutions against unmixed solutions. The spectra were registered at increasing concentrations of ADA. Inset, Scatchard plot for interaction of DPPII and ADA.

high molecular weight ADA (~200 kD) isolated by Lindley and Pisoni from human fibroblast lysosomes [32]. They discussed particularly the probable importance of lysosomal compartmentalization of ADA for maintenance of cellular energy at low cytosolic adenosine level. This suggestion might be considered as one of the working hypotheses for elucidation of the unknown physiological significance of binding of DPPII and ADA.

Further investigations should be directed to these objectives and to the elucidation of binding centers responsible for the interaction of DPPII with ADA.

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REFERENCES

1. Yaron, A., and Naider, F. (1993) *Crit. Rev. Biochem. Mol. Biol.*, **28**, 31-81.
2. Cunningham, D., and O'Connor, B. (1997) *Biochim. Biophys. Acta*, **1343**, 160-186.
3. Rosenblum, J., and Kozarich, J. (2003) *Curr. Opin. Chem. Biol.*, **7**, 496-504.
4. Mentlein, R., and Struckhoff, G. (1989) *J. Neurochem.*, **52**, 1284-1293.
5. Chen, W.-T., Kelly, T., and Ghersi, G. (2003) *Curr. Top. Devel. Biol.*, **54**, 207-232.
6. Busek, P., Malik, R., and Sedo, A. (2004) *Int. J. Biochem. Cell. Biol.*, **36**, 408-421.
7. McDonald, J., Leibach, F., Grindeland, R., and Ellis, S. (1968) *J. Biol. Chem.*, **243**, 4143-4150.
8. Fukasawa, K. M., Fukasawa, K., Higaki, K., Shiina, N., Ohno, M., Ito, Sh., Otogoto, J., and Ota, N. (2001) *Biochem. J.*, **353**, 283-290.
9. Maes, M.-B., Lambeir, A.-M., Gilany, K., Senten, K., van der Veken, P., Leiting, B., Augustins, K., Scharpe, S., and de Meester, I. (2005) *Biochem. J.*, **386**, 315-324.
10. McDonald, J. K., and Schwabe, C. (1980) *Biochim. Biophys. Acta*, **616**, 68-81.
11. Maes, M.-B., Martinet, W., Schrijvers, D. M., van der Veken, P., de Meyer, G. R. Y., Augustyns, K., Lambeir, A.-M., Scharpe, S., and de Meester, I. (2006) *Biochem. Pharmacol.*, **72**, 70-79.
12. Hagihara, M., Mihara, R., Togari, A., and Nagatsu, T. (1987) *Biochem. Med. Metab. Biol.*, **37**, 360-365.
13. Maes, M. B., Scharpe, S., and de Meester, I. (2007) *Clin. Chim. Acta*, **380**, 31-49.
14. Gorrell, M., Gysbers, V., and McCaughan, G. (2001) *Scand. J. Immunol.*, **54**, 249-264.
15. Boonacker, E., and van Noorden, C. (2003) *Eur. J. Cell Biol.*, **82**, 53-73.
16. Kameoka, J., Tanaka, T., Nojima, Y., Schlossman, S. F., and Morimoto, Ch. (1993) *Science*, **261**, 466-469.
17. Daddona, P., and Kelley, W. N. (1978) *J. Biol. Chem.*, **253**, 4617-4623.
18. Van der Weyden, M., and Kelley, W. (1976) *J. Biol. Chem.*, **251**, 5448-5456.
19. Mardanyan, S., Sharoyan, S., Antonyan, A., Armenyan, A., Cristalli, G., and Lupidi, G. (2001) *Biochim. Biophys. Acta*, **1546**, 185-195.
20. Weber, K., and Osborn, M. (1969) *J. Biol. Chem.*, **244**, 4406-4412.
21. Bradford, M. (1976) *Analyt. Biochem.*, **72**, 259-268.
22. Murphy, J., and Kies, M. (1960) *Biochim. Biophys. Acta*, **45**, 382-384.
23. Davies, R., Edwards, P., Watts, H., Lowe, C., Buckle, P., Yeung, D., Kinning, T., and Pollard-Knight, D. (1994) *Techniques in Protein Chemistry*, Academic Press, San Diego, pp. 285-292.
24. Kakehi, K., Oda, Y., and Kinoshita, M. (2001) *Analyt. Biochem.*, **297**, 111-116.
25. Leatherbarrow, R. J. (2001) *GraFit Version 5 Erithacus Software Ltd.*, Horley, U. K.
26. Schrader, W. P., and Stacy, A. R. (1977) *J. Biol. Chem.*, **252**, 6409-6415.
27. Huang, K., Takagaki, M., Kani, K., and Ohkubo, I. (1996) *Biochim. Biophys. Acta*, **1290**, 149-156.
28. Sharoyan, S., Antonyan, A., Mardanyan, S., Lupidi, G., and Cristalli, G. (2006) *Acta Biochim. Polon.*, **53**, 539-546.
29. Weihofen, W. A., Liu, J., Reutter, W., Saenger, W., and Fan, H. (2004) *J. Biol. Chem.*, **279**, 43330-43335.
30. Richard, E., Arrendondo-Vega, X., Santisteban, I., Kelly, S., Patel, D., and Hershfield, M. (2000) *J. Exp. Med.*, **192**, 1223-1235.
31. Dong, R. P., Tachibana, K., Hegen, M., Munakata, Y., Cho, D., Schlossman, S., and Morimoto, C. (1997) *J. Immunol.*, **159**, 6070-6076.
32. Lindley, E., and Pisoni, R. (1993) *Biochem. J.*, **290**, 457-462.