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Biophysical Chemistry 75 (1998) 129–139

Biophysical Chemistry

The relationship between biological activity and the electronic structure and transfer of the whole acidic PLA_2 molecule in ab initio level

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Received 27 April 1998; accepted 19 August 1998

Abstract

The electronic structure of the whole molecule of acidic phospholipase A_2 (PLA_2) from the venom of *Agkistrodon halys pallas* (*A. halys pallas*) has been calculated using the extended negative factor counting (ENFC) method in which dimers were calculated at the ab initio level using a minimal basis set, with simulation of the aqueous environment. Hopping conductivities were determined by the use of random walk theory. The results show that the frontier orbitals are mainly localized to residues which are involved in the biological activity of acidic PLA_2. The C-terminal region might play some important role in biological activity because of its active electrons. The aromatic patch on the surface of the enzyme, together with two neighbouring acidic residues, has very active electrons that may be responsible for the inhibition of platelet aggregation. Trp30, which is involved in the interfacial recognition region, may transfer its electrons to the aggregated substrate. It is also concluded that the conductivity of the protein is caused mainly by holes transported through the valence band rather than electrons transferred in the conductive band. The a.c. conductivity of acidic PLA_2 confirms that proteins, if doped, are amorphous conductors. Moreover, the a.c. conductivities of acidic PLA_2 are approximately one order of magnitude higher than those of some other proteins. This suggests that the toxicity of acidic PLA_2 may be related to its high a.c. conductivity.

Keywords: Phospholipase A_2 (PLA_2); Electronic structure; a.c. Conductivity; Biological activity; Frontier orbital

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Abbreviations: *A. halys pallas*, *Agkistrodon halys pallas*; ENFC, extended negative factor counting; PLA_2, phospholipase A_2; HOMOs, highest occupied molecular orbitals; LUMOs, lowest unoccupied molecular orbitals; IRS, interfacial recognition site.

0301-4622/98/$ - see front matter
PII S0301-4622(98)00200-2

1. Introduction

Phospholipase A_2 (PLA_2s) are ubiquitous enzymes that are present in most cells and tissues. PLA_2s catalyze the hydrolysis of the Sn-2 ester bond of phosphoglycerides, releasing free fatty acid with concomitant formation of lysophospholipids [1–5]. PLA_2s play important roles in diverse cellular processes including phospholipid digestion, metabolism and signal transduction. They also initiate the biosynthesis of inflammatory and allergic mediators, eicosanoids and platelet-activating factors [6].

The venom of *Agkistrodon halys pallas*, found in the Zhejiang and Jiangsu province of China, contains three phospholipase A_2 species, an acidic PLA_2 with an isoelectric point of 4.5 and neutral and basic PLA_2 with isoelectric points of 6.9 and 9.3, respectively [7]. They differ broadly in enzymatic activity, pharmacological activity and lethal potency [8,9]. Among these three species, the acidic PLA_2 possesses the highest enzymatic activity and the weakest toxicity. Moreover, the acidic PLA_2 displays the ability to inhibit platelet aggregation, while the neutral and the basic PLA_2 do not [10,11]. It has been reported that the acidic PLA_2 from the venom of *A. halys pallas* can increase cAMP concentration by interacting with the platelet cytomembrane, hence inhibiting the process of platelet aggregation [12]. Some experiments suggested that the functional site for the inhibition of platelet aggregation is separate from the catalytic site because there seems to be no relationship between enzymatic activity and the inhibition of platelet aggregation [10]. However, little is known about the detailed mechanism of the inhibition of platelet aggregation.

PLA_2 is far more active toward aggregated than single substrate molecules [13,14]. Many experiments have been designed to elucidate this feature of the action of PLA_2. Some support the hypothesis that the properties of the aggregated substrate are key factors for enzyme activity [15,16]. Others explain the effect by a conformational change in the enzyme as a result of binding to the substrate layer [17]. As it is unlikely that the crystal structure of a PLA_2-aggregated substrate complex can be determined, it will be difficult to experimentally reveal the details of the interaction between them.

Theoretical calculations based on an entire protein molecule can determine the electronic structure and energy levels which may play important roles in the expression of its enzymatic and biochemical activities and pharmacological effects [18–22]. Therefore an investigation of the electronic structure of the acidic PLA_2 from the venom of *A. halys pallas* will provide valuable insights into its biological activities. Recently, some ab initio methods [23,24] that can be performed on an entire macromolecule, such as the extended negative factor counting (ENFC) method [23] of Ye and Ladik and the linear scaling algorithm [24] of Challacombe and Schwegler have been developed. Studies on periodic and aperiodic models have shown that the activity of both protein and DNA may be partially explained by their electronic transport [25]. Ye and Ladik [18–21] have successfully calculated the electronic structure of the whole molecule of pig insulin and described the electronic mechanism of trans-membrane signal transduction by insulin and its receptor.

The conductivity of enzymes have been studied, both experimentally and theoretically, because electron transport is involved in enzyme-catalyzed reactions in many cases. It has been shown that proteins (including enzymes) can act as semiconductors rather than insulators [26]. Some theoretical studies showed that the conductivity of proteins is at least partially due to the hopping of electronic charge carriers along the main chains of these proteins [27–30]. The first example of protein conductivity was calculated by Ye and Ladik [18,31]. They concluded that a native protein was a good amorphous conductor with high conductivity in the high frequency range, corresponding to the time period of biochemical reactions, if the direction of the effective electric field was within a small angle of the direction of a chain segment.

The unresolved questions about the activity of the acidic PLA_2 from the venom of *A. halys pallas* lead us to pursue these matters by theoretical studies. In this paper, the electronic structure and hopping conductivity of the whole acidic

PLA_2 molecule were calculated using the SCF method in ab initio level. A minimal basis set was used in the calculations, with point charges around the charged residues simulating the solution environment.

2. Methods

Fig. 1 shows the sequence of the acidic PLA_2 from venom of *A. halys pallas*. Recently, the crystal structure of this enzyme has been determined [32]. The enzyme contains 124 residues, and has three α-helices and a antiparallel β-sheet known as a β-wing. Seven disulfide bonds make the conformation of the enzyme very stable. The coordinates of the crystal structure of this enzyme were used in the calculations, with the hydrogen atom coordinates determined theoretically. All 1853 atoms, including hydrogen atoms, of acidic PLA_2 were involved in the quantum chemical calculations. Clementi minimal basis set was applied in the calculations and the numbers of basis functions used to determine the electronic structure and conductivity of the entire molecule was 5801.

Some approximations [23,24] have been developed for calculations on whole protein molecules because protein molecules are so large that they can not be calculated by full SCF ab initio methods. We employed an extended negative factor counting (ENFC) method which was developed by Ye [23] based on the NFC theory [33–35]. The whole protein molecule is divided into dimers which consist of two amino acid residues with chemical bonds (including disulfide bonds) in their three-dimensional conformation. Pseudo-atoms

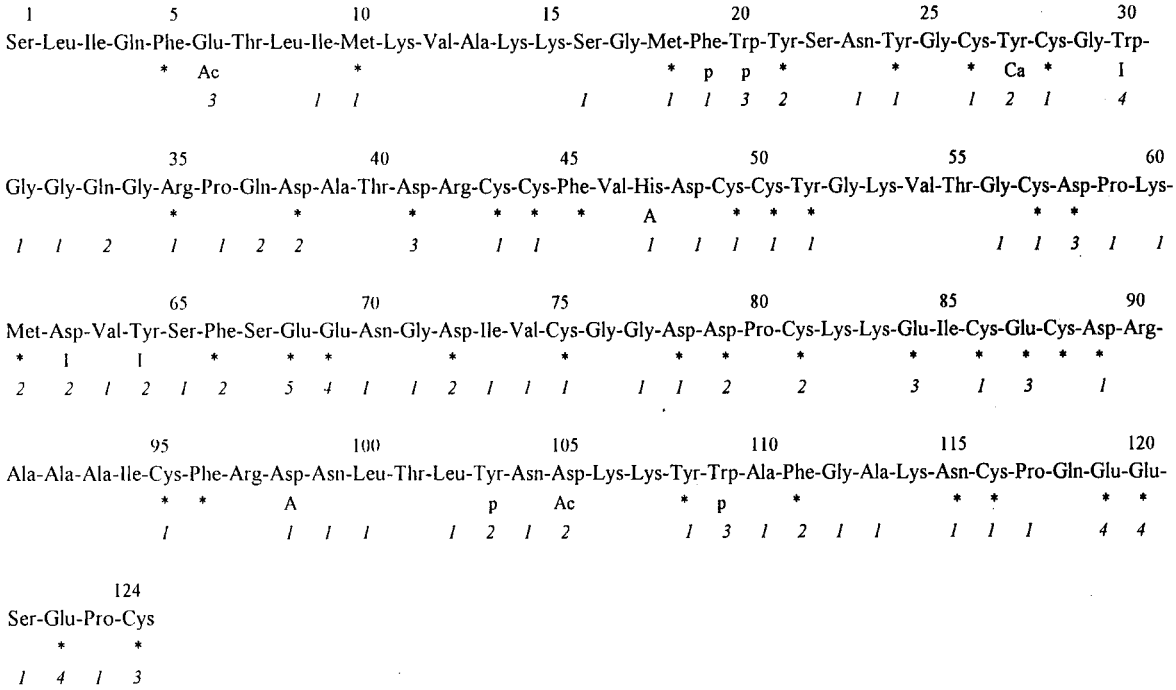


Fig. 1. The Primary sequence of acidic PLA_2 from the venom of *A. halys pallas*. (p) Represent the frontier orbitals which are localized mainly on these aromatic patch area residues; (Ca) represents the frontier orbital which is localized mainly on the residue involved in Ca-binding area; (I) represents the frontier orbitals which are localized mainly on these IRS area residues; (A) represents the frontier orbitals which are localized mainly on active site residues; (Ac) represents the frontier orbitals which are localized mainly on these acidic residues nearby the aromatic patch; and (*) represent the frontier orbitals which are localized mainly on those residues being inactive. The *italic numbers* below the different residues give the number of hopping centers taken into account in calculation of a.c. conductivities.

are added to simulate the chemical environment at the end of each dimer. In order to simulate the aqueous environment, point charges are put around the residues that have net electric charges. Each dimer is used for calculations by the ab initio SCF LCAO method to obtain their Fock and overlap matrices. Based on these dimer matrices, the Fock and overlap matrices of the whole protein molecule are constructed. Energy levels and wave functions of the entire protein molecule can then be calculated using the constructed Fock and overlap matrices; for details, see Ye and Ladik [19].

The conductivities of the acidic PLA_2 from the venom of *A. halys pallas* were determined on the basis of the primary jump rates calculated using the energy levels and wave functions obtained above. In this paper, the acidic PLA_2 is also treated as a quasi-one-dimensional amorphous system, and the random-walk theory of Odagaki and Lax [36] is applied to calculate its conductivity based on the Einstein relationship

$$\sigma(\varpi) = \frac{n_v e^2}{kT} D(\varpi) \quad (1)$$

where n_v is the number of effective charge carriers in a unit volume, e is the electronic charge, k is the Boltzman constant and T is the absolute temperature. See Ye and Ladik [31] for details of the formulae.

3. Results and discussion

3.1. Frontier orbitals

Table 1 lists the highest 40 occupied molecular orbitals (HOMOs) and Table 2 lists the lowest 40 unoccupied molecular orbitals (LUMOs). From the two tables, one can see that most of the frontier orbitals are localized to an individual residue although some frontier orbitals have wavefunctions that are mainly distributed over disulfide or peptide bonds. The highest three filled and lowest three unfilled molecular orbitals are localized on residues which are involved in the biological activities of the enzyme. The two tables show that 25% of HOMOs and 35% of LUMOs correspond to residues which have been shown experimentally to have biological activity. These results support the conclusion, drawn previously by Ye and Ladik, that the frontier orbitals of a protein can influence its biochemical activity [19,21,22]. Moreover, from the tables, one can predict that there may be, as yet, unknown biological functions which would be mediated by the other residues that also have HOMOs and/or LUMOs.

The highest two occupied molecular orbitals and the lowest and third lowest unoccupied molecular orbital correspond to residues in the 'aromatic patch' of PLA_2. This indicates that these aromatic residues have the highest electronic activity. The aromatic patch, which does not exist in neutral or basic PLA_2 [32], is located on the outer surface of acidic PLA_2 and contains four aromatic residues: Phe19, Trp20, Phe103 and Trp109. It has been proposed that this aromatic patch may be relevant to the inhibition of platelet aggregation [32], but no experimental evidence is currently available. It is notable that most PLA_2s that inhibit platelet aggregation are acidic proteins [8,10,37], therefore negatively charged groups (such as acidic residues) may be important for this function. The residues Glu6 and Asp105, which correspond to the sixth and the 32nd HOMOs, respectively, are located close to the aromatic patch. The aromatic patch and these two nearby acidic residues form a structure which is common to the two other PLA_2 species that inhibit platelet aggregation (the acidic PLA_2 from *A. halys blomhoffii* and the acidic PLA_2 from T. gramineu) [32]. It can be seen that four HOMOs and eight LUMOs (Tables 1 and 2) belong to the residues of this structure making it an area that will be active in electronic processes. Therefore this structure forms a specific site that may interact with the platelet membrane and electronic transfer should be an important factor in this interaction.

From the tables, one can also see that the third HOMO and the second LUMO are localized to Trp30 which has been suggested to be involved in interfacial recognition. PLA_2 species can catalyze substrate aggregates with higher rates than monomeric substrates. It has been proposed that

Table 1
The highest 40 occupied molecular orbitals (HOMOs)

	Numbering	Energy levels (eV)	Positions of the wave-functions	Activity
1	3708	−9.9814	Trp20	Aromatic patch
2	3707	−10.0967	Trp109	Aromatic patch
3	3706	−10.1242	Trp30	IRS
4	3705	−10.5269	Glu120	Acidity
5	3704	−10.5474	Tyr21	
6	3703	−10.5477	Glu6	Aromatic patch
7	3702	−10.5495	Glu69	
8	3701	−10.5612	Glu68	
9	3700	−10.5658	Met61	
10	3699	−10.5806	Glu122	
11	3698	−10.5890	Cys57–Cys81	
12	3697	−10.6138	Cys49–Cys124	
13	3696	−10.6939	Met18	
14	3695	−10.7199	Tyr27	Ca-binding area
15	3694	−10.7200	Glu119	
16	3693	−10.7387	Tyr108	
17	3692	−10.7480	Asp41	
18	3691	−10.7672	Glu84	
19	3690	−10.7727	Glu87	
20	3689	−10.7995	Tyr64	
21	3688	−10.8307	Cys26–Asn115–Cys116	
22	3687	−10.8307	Met10	
23	3686	−10.8557	Cys43–Cys95	
24	3685	−10.8661	Cys49–Cys124	
25	3684	−10.8849	His47–Tyr51	
26	3683	−10.8889	His47–Tyr51	Active site
27	3682	−10.8918	Cys75–Cys86	
28	3681	−10.9065	Asp72	
29	3680	−10.9122	Tyr103	
30	3679	−10.9166	Tyr24	
31	3678	−10.9356	Asp58	Acidity patch
32	3677	−10.9427	Asp105	
33	3676	−10.9615	Asp38	
34	3675	−10.9651	Asp62	
35	3674	−10.9688	Asp78–Asp79	
36	3673	−10.9862	Asp78–Asp79	
37	3672	−11.0041	Asp89	
38	3671	−11.0147	Asp98	Active site
39	3670	−11.0227	Glu122–Pro123	
40	3669	−11.0481	Gly71–Asp72	

an interfacial recognition site (IRS), which binds with the ordered substrate, exists on the surface of PLA_2s and is located around the entrance of the active site cleft [1,38]. According to the crystal structure [32], Trp30 is an important part of the IRS and located at the entrance of the hydrophobic channel connected to the active site. Its aromatic ring may insert into the aggregated substrate as an anchor, making the enzyme bind more tightly to the substrate. Our quantum chemical results indicate that Trp30 has very active electrons which may have an important effect on the interaction of the enzyme with the aggregated substrate. Electronic transport might occur between Trp30 and the substrate aggregates when the enzyme interacts with the ordered substrates.

Table 2
The lowest 40 unoccupied molecular orbitals (LUMOs)

	Numbering	Energy levels (eV)	Positions of the wave-functions	Activity
1	3709	1.2256	Trp109	Aromatic patch
2	3710	1.2499	Trp30	IRS
3	3711	1.4525	Trp20	Aromatic patch
4	3712	1.7074	Tyr51	
5	3713	1.7601	Phe96	
6	3714	1.8285	Tyr103	Aromatic patch
7	3715	1.8343	Phe96	
8	3716	1.8873	Tyr24	
9	3717	1.9205	Tyr27	Ca-binding area
10	3718	1.9867	Phe45	
11	3719	2.0036	Tyr108	
12	3720	2.0433	Tyr64	
13	3721	2.0582	Phe45	
14	3722	2.0675	Phe19	Aromatic patch
15	3723	2.0849	Tyr21	
16	3724	2.1681	Phe5	
17	3725	2.2032	Phe19	Aromatic patch
18	3726	2.2235	Tyr103	Aromatic patch
19	3727	2.2428	Phe5	
20	3728	2.2434	Phe111	
21	3729	2.2827	Tyr27	Ca-binding area
22	3730	2.2848	Phe66	
23	3731	2.3291	Phe66	
24	3732	2.3375	Tyr51	
25	3733	2.4005	Phe111	
26	3734	2.4540	Tyr24	
27	3735	2.5433	Tyr108	
28	3736	2.5681	Tyr64	
29	3737	2.5713	Tyr21	
30	3738	2.8921	Trp109	Aromatic patch
31	3739	2.9816	Trp30	IRS
32	3740	2.9857	Cys57–Cys81	
33	3741	3.2070	Trp20	Aromatic patch
34	3742	3.2078	Cys50–Cys88	
35	3743	3.2167	Arg35	
36	3744	3.2735	Cys28–Cys44	
37	3745	3.3112	Ser1–Leu2–Ile3	
38	3746	3.3740	Cys26–Lys114–Asn115–Cys116–Pro117	
39	3747	3.3767	Cys43–Cys95	
40	3748	3.3776	Cys49–Cys124	

Another important part of the IRS is the N-terminal α-NH_3^+ group and its surrounding region. In the acidic PLA_2 from venom of *A. halys pallas*, the N-terminal α-amino group is linked via a hydrogen bonding system to the 59–64 loop. This hydrogen bond system involves Ser1, Leu2, Glu4, Pro59, Lys60, Asp62 and Tyr64 and is very important for the activity of the enzyme on ordered substrates [32]. From the tables and Fig. 1, one can see that, with the exception of Asp62 and Tyr64, all the residues involved in this hydrogen bonding system do not have frontier orbitals. This indicates that electronic transfer is not an important factor when this part of the IRS interacts with the aggregated substrates.

His47 and Asp89 are localized within the first

40 HOMOs (Table 1), indicating that it is easy for His47 and Asp89 to take part in biochemical reactions to exert their biological activity. As has been shown, the key residues involved in the catalytic hydrolysis reaction of the acidic PLA_2 from venom of *A. halys pallas* are His47, Asp89 and one water molecule [32]. The water molecule is rendered more nucleophilic by transferring a proton to the imidazole of His47, and then it attacks the carbonyl carbon of the substrate. The ϵ-N atom of His47 is in close contact with the carboxylate oxygens of Asp89 so that the positive charge of His47 is stabilized by Asp89. Our quantum chemical results showing that His47 and Asp89 can easily transfer electrons is in agreement with the catalytic activity of the enzyme.

In addition, of all the Ca^{2+}-binding loop residues, only Trp27 appears in the first 40 HOMOs and LUMOs indicating that these residues are chemically inert. That is, apart from Trp27, they can not readily take part in chemical reactions in which electron transfer is involved, and their function is to stabilize the three-dimensional conformation of the enzyme.

3.2. The hopping conductivity

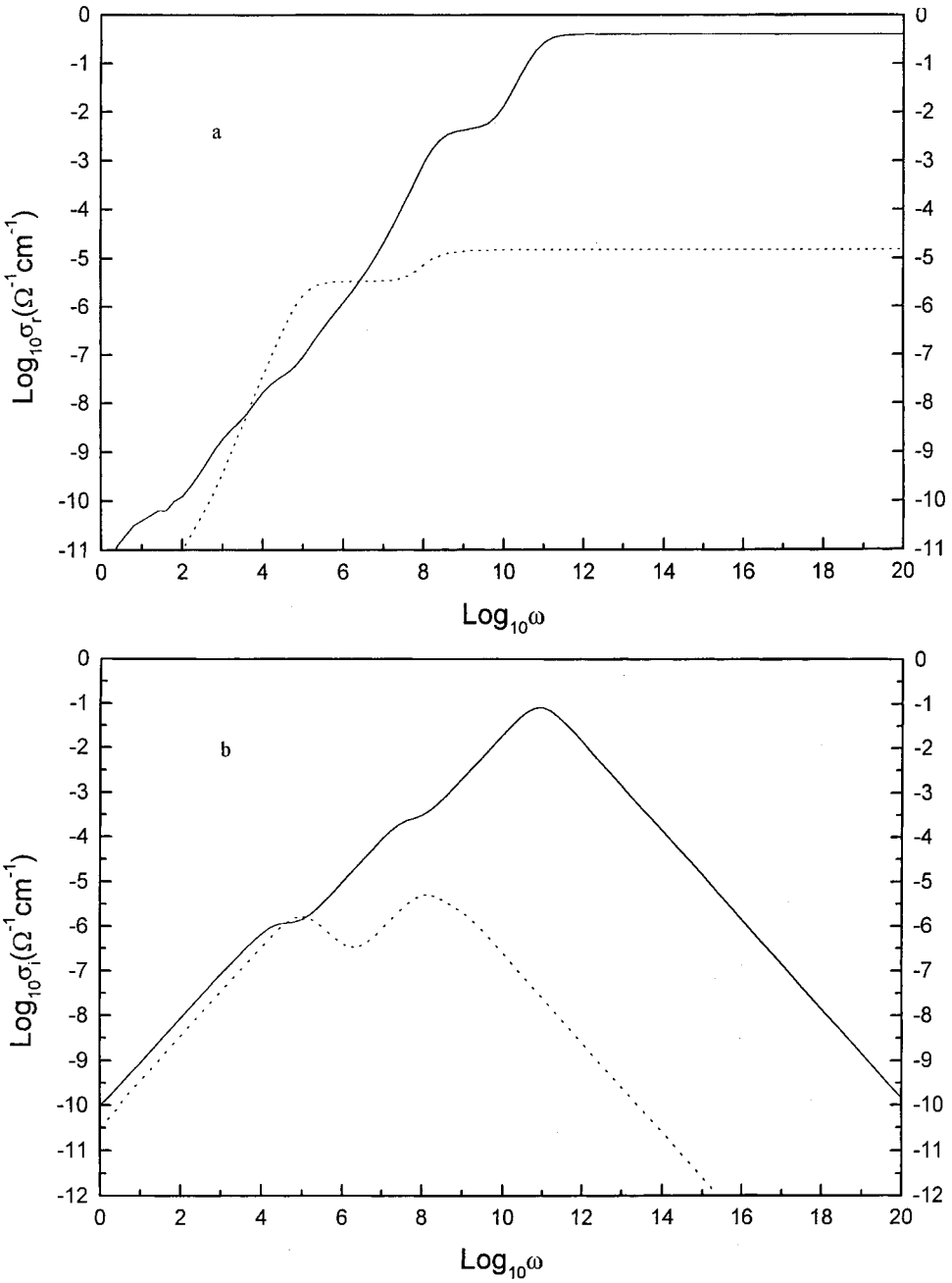
On the basis of the energy levels and wave functions obtained above, the a.c. conductivities of the acidic PLA_2 from the venom of *A. halys pallas* were calculated. In this paper, the acidic PLA_2 molecule is treated as a quasi-one-dimensional disordered finite system. The formulae of Odagaki and Lax [36] have been applied to numerically calculate its a.c. hopping conductivity. As an approximation, only those hopping events which take place between first and second neighbour residues have been taken into account. For details of all the formulae and approximations in the calculation of hopping conductivity, see Ye and Ladik [31].

We have performed three calculations of conductivity. The first one involves only the highest 200 filled orbitals. The second one involves only the lowest 200 unfilled orbitals. The last one is performed using both the highest 200 filled orbitals and the lowest 200 unfilled orbitals. Because the highest filled orbitals are regarded as a valence band and the lowest unfilled orbitals are regarded as a conductive band, we call the conductivities calculated using the highest 200 filled orbitals and the lowest 200 unfilled orbitals as valence band conductivity and conductive band conductivity, respectively. In general, the valence band of a protein is fully occupied while the conductive band of a protein is completely empty. When a protein is doped, such as by photoexcitation or a biochemical reaction, electrons can be taken out from the valence band leaving holes. These holes are very active and can be transported freely in the valence band. If the holes are subject to a force by an external electric field, they can be transferred along the direction of the field to form the conductivity of protein. Therefore the charge carriers are holes in the valence band. Similarly, when a protein is doped, there may be electrons given to the conductive band. These electrons are also active and can be transported in the conductive band. They can also form an electric current under the force of an external field. So the charge carriers are electrons in the conductive band. In each calculation, 100 charge carriers have been considered and the volume of the PLA_2 molecule is 32 480 $Å^3$ [39].

The calculated a.c. conductivities are shown in Fig. 2. From this figure, we can see that the a.c. conductivities increase with the frequency ω, as in the case of disordered inorganic solids. This confirms the conclusion that a protein is a good amorphous conductor [18,20–22].

Fig. 2a,b give the real and imaginary parts of the a.c. conductivities, respectively. The combination of the real and imaginary parts makes the curves of the absolute value of the a.c. conductivities smoother (see Fig. 2c). From Fig. 2c, one can see that the absolute value of the valence band conductivity (solid line) increases with the frequency, ω, in the range of $0 < \omega < 10^{11}$, and reaches a constant value in the high frequency range, $\omega > 10^{11}$. The absolute value of the conductive band conductivity (dotted line) increases when ω is $< 10^8$, and reaches a constant value after $\omega = 10^8$. From Fig. 2c, we can also see that the absolute value of the valence band conductivity is more than four orders of magnitude higher than that of the conductive band conductivity. A

conclusion can be drawn that the a.c. conductivity of the protein is produced mainly by the valence band. In addition, another conductivity–frequency curve was calculated using the highest 200 filled orbitals and the lowest 200 unfilled orbitals. This curve is almost identical and overlays the curve of valence band conductivity (solid line) so that it can not be distinguished in Fig. 2. Because the conductivity calculated with both filled and unfilled orbitals is similar to the valence band con-



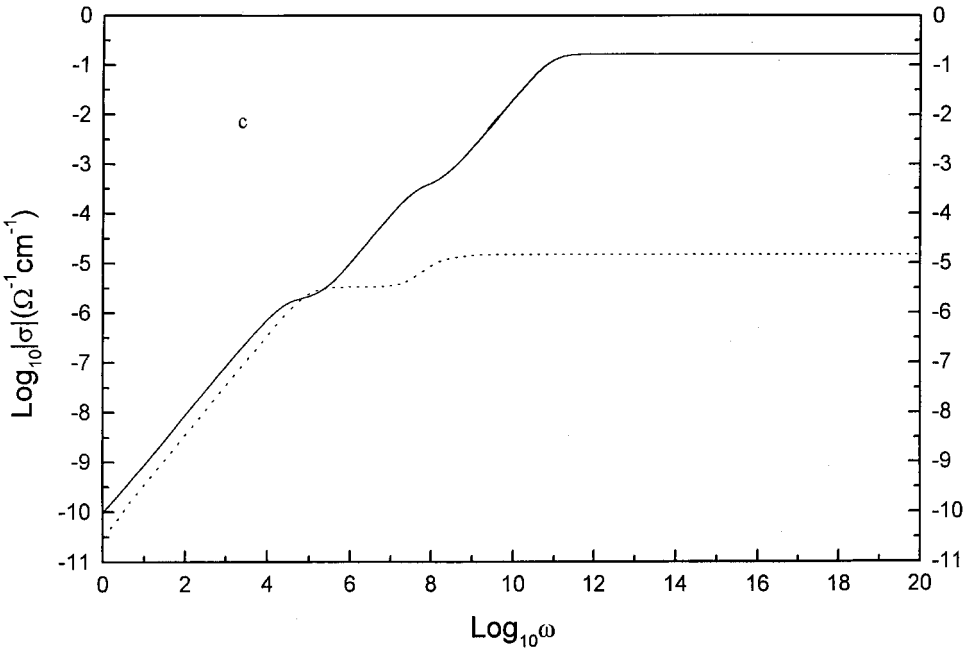


Fig. 2. The a.c. conductivities of acidic PLA_2 from the venom of *A. halys pallas*. (a) The real parts of a.c. conductivities. (b) The imaginary parts of a.c. conductivities. (c) The absolute values of a.c. conductivities. The solid line (———) represents the curve which is calculated involving 200 HOMOs. The dot line (· · ·) represents the curve which is calculated using 200 LUMOs.

ductivity, we can draw the same conclusion as above that the a.c. conductivity of a protein is produced mainly by the valence band and the contribution of the conductive band can be ignored. That is, the electron transport in a protein is controlled mainly by holes transported through the valence band.

The number of hopping centers in a residue taken into account in the calculation of a.c. conductivity are shown in Fig. 1 using *italic* numbers below the residues. It can be observed that there are two regions, as well as Trp30, that have many more centers than the others. One region is located at the C-terminus, the other is at residues 68–69. Therefore electronic transport mainly happens in these regions. Based on the crystal structure of the enzyme, one can see that a complex interaction system is formed between the C-terminal region and the Ca^{2+}-binding loop. The C-terminal region appears to contribute to the stability of the Ca^{2+}-binding area. As the C-terminal region is one of the main paths of electron transport and it also has a specific position in the enzyme, we can predict that the C-terminal region may play some important role in the activity of the enzyme. In addition, Trp30 has been suggested to be involved in the interfacial interaction with the aggregated substrate. As the third HOMO and the second LUMO are located on Trp30, it was suggested, above, that Trp30 might transfer electrons to the substrates when acidic PLA_2 binds to ordered substrates. The result here, that Trp30 has many more hopping centers, supports the above conclusion.

Ye and Ladik have reported that the a.c. conductivity of active is higher than that of inactive lysozyme [21,31]. They have also calculated the a.c. conductivities of pig and hagfish insulin [20–22]. The conductivities of these proteins are shown in Table 3. A comparison of these reported a.c. conductivities with that of the acidic PLA_2 from venom of *A. halys pallas* shows that the acidic PLA_2, which is toxic, has a much higher a.c. conductivity by at least one order of magnitude. This means that the enzyme can rapidly transfer electrons and so has high electronic ac-

Table 3
The conductivities of proteins which have been reported and calculated here

	Conductivity (Ω^{-1} cm^{-1})	Ref.
Pig insulin I	2.51×10^{-2}	[20,22,31]
Pig insulin II	1.78×10^{-4}	[20,22]
Hagfish insulin	1.99×10^{-3}	[22]
Native lysozyme	1.00×10^{-3}	[21,31]
Inactive lysozyme	3.98×10^{-6}	[21]
Native subtilisin	5.62×10^{-5}	[21]
Inhibited subtilisin	6.31×10^{-3}	[21]
Acidic PLA_2	1.680×10^{-1}	This paper

tivity. It can be proposed that high a.c. conductivity, as seen in this enzyme, might be a feature of toxic proteins.

4. Conclusions

The electronic structure and a.c. conductivity have been calculated by the ENFC method and random walk theory on the entire molecule of the acidic PLA_2 from the venom of *A. halys pallas*. The calculations revealed that the frontier orbitals mainly involve residues which are important for the biological activity of the enzyme. Several frontier orbitals among the first 40 HOMOs and LUMOs localize to residues of the aromatic patch on the surface of the enzyme and the two nearby acidic residues. This unique structure forms a specific site which might interact with the platelet membrane and electronic interaction and transfer should be an important factor in the inhibition of platelet aggregation. PLA_2s are more active on an aggregated substrate than a monomeric substrate. The interfacial recognition site of the enzyme is believed to interact with substrate aggregates. As crystals of a PLA_2-aggregated substrate complex have not yet been grown, little is known about the details of the interfacial interaction. In our calculation results, the electrons of Trp30, which is a residue of the interfacial recognition site, are so active that there may be electron transport between Trp30 and the aggregated substrate. More hopping centers were found on the C-terminal region of the protein, suggesting that this region is one of the main paths of electron transport. Therefore it was predicted that the C-terminal region may play some important role in the activity of the enzyme. From the results on a.c. conductivity, a conclusion can be drawn that the a.c. conductivity of a protein may be produced mainly by holes transported through the valence band. Moreover, the high a.c. conductivities of the acidic PLA_2 might be responsible for its toxicity.

Acknowledgements

The authors are grateful for helpful discussions with Prof. Z. Lin of the Institute of Biophysics, Chinese Academy of Sciences, for the financial support from the 'National Natural Scientific Foundation of China' (project number: 29473146), the 'Chinese High Performance Computing Foundation' (project number: 96108), the 'Chinese High Technology Development Projects' (project number: 863-306-ZD-01-4) and the 'Chinese Pangdeng Projects', and for the use of the Power-Challenge/R10000 computer of the 'Computer Network Information Center of the Chinese Academy of Sciences', the Dawn-1000 computer of the 'National Research Center for Intellectual Computing System of the Chinese Academy of Sciences' and the IBM/SP2 computer of the 'China Education and Research Network Center'.

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