A molecular model for interfacial activation in phospholipase A_2

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Received 20 December 1996; revised version received 27 January 1997

Abstract Electrostatic calculations predict that amino-terminal conformation and ionisation contribute significantly to transition state stability in phospholipase A_2, so that control of these factors by binding to aggregated substrate provides a plausible mechanism for interfacial activation. In particular, it is suggested that a part of the pH dependence of interfacial activity may arise from transient deprotonation of an ordered amino-terminus. Interface charge and the detailed structure of the interfacial complex are also predicted to influence catalytic activity. The model is compared with available biochemical data.

Key words: Interfacial activation; Electrostatics; Phospholipase A_2; Conformational change; Molecular modeling

1. Introduction

Phospholipases A_2 (PLA_2s) hydrolyse fatty acids esterified at the *sn*-2 position, and are involved in a variety of physiological processes [1]. The relatively small (around 14 kDa), calcium-dependent, secretory classes I and II PLA_2s [2] have been widely studied with regard to the phenomenon of interfacial activation (IA), whereby significantly increased activity is exhibited with aggregated versus monomeric substrates [3]. Biochemical [4] and X-ray crystallographic [5,6] studies on pancreatic mature, transaminated and pro-PLA_2s have demonstrated that an ordered amino-terminus (N-t) mediates interfacial binding. Crystallographically determined structures of mature, non-interfacially bound enzymes show an ordered N-t [2], whilst solution structures of non-interfacially bound enzymes show an ordered [7] or disordered [8] N-t, and an ordered N-t in the ternary complex [8]. The role of the N-t in IA is unclear [7]. We report calculations of charge interactions between the N-t and the putative transition state (TS), and suggest a model for a significant contribution to IA in PLA_2 catalysis at a neutral interface. Further calculations indicate that both interfacial charge and the detailed structure of the interfacial complex could also play significant roles in IA for PLA_2.

2. Methods

The TS complex [5,9] was modelled from an inhibitor-porcine pancreatic PLA_2 structure [10]. A previous discussion of this modelling [9] indicates that it probably provides a reasonable basis for electrostatic calculations based on dipolar TS charge. Electrostatic calculations of TS stabilisation were made with finite difference solutions to the Poisson-Boltzmann equation [11], implemented in the program FDCALC. Relative dielectric values of 3 for protein/interfacial slab and 80 for solvent were used, with an ionic strength of 0.15 M. A boundary condition of zero electrostatic potential was enforced at the edge of a solvent layer that extended at least 10 Å from all low dielectric regions. Boundary errors between calculations were minimised by extending all finite difference grids to the size of that used for the calculation with the largest enzyme-interfacial slab separation. Dipolar TS charge (Fig. 1) interacts with the enzyme charge at neutral pH with standard pK_a values. Partial charges were included with the GROMOS charge set [12]. Molecular graphics and manipulation, including the study of N-t deletions, was performed with QUANTA (Molecular Simulations Inc.) running on a Silicon Graphics workstation. A cylindrical interfacial slab was constructed with a radius of 34 Å and a depth of 30 Å, thereby providing a suitable model for PLA_2 at a micellar interface (Fig. 3). Interfacial charge density (σ_{int}) was modelled with a set of point charges at 3 Å spacing in the interfacial plane, and placed either on the outer slab surface, or just outside the enzyme envelope in cases of slab/enzyme overlap. These charges were scaled to reproduce a surface density of -1 *e*/77 Å^2, modelling an anionic phospholipid. Calculation of $\Delta\Delta G_{cs}^{TS}$ shows a change in TS electrostatic stabilisation (ΔG_{cs}^{TS}) due to a specific process, such as N-t protonation or interfacial binding.

3. Results and discussion

Fig. 1 shows a model for the dipolar TS in porcine pancreatic PLA_2 [9], constructed from an enzyme-inhibitor complex [10] and the proposed catalytic mechanism [5]. Stabilisation of the TS in this model derives from favourable interactions with $H48^+$ and the oxyanion [13]. The ordered N-t lies adjacent to the active site, giving electrostatic interactions with the TS due both to the net charge of the protonated N-t, and to the effect of the N-t in plugging its binding site within the enzyme, effectively increasing TS charge burial. Fig. 2A shows these 2 terms, with calculations of differences in TS electrostatic stabilisation ($\Delta\Delta G_{es}^{TS}$) as an ordered and protonated N-t is first deprotonated and then disordered. The disordered N-t was modelled with removal of the first 2 amino acids (AL) and the sidechain of W3, following the disruption reported for the transaminated enzyme [6]. Deprotonation of the N-t relieves the unfavourable $N\text{-}t^+\text{-}H48^+$ interaction, leading to a calculated TS stabilisation of 8.5 kJ/mol. Disordering of the deprotonated N-t is calculated to destabilise the TS by 9.3 kJ/mol. This term arises from a reduction in favourable interactions between the TS and other PLA_2 charges, particularly $D99^-$, consistent with reduced charge burial (Fig. 1).

The N-terminal states of Fig. 2A are a subset of those which can be defined in relation to PLA_2 N-t protonation/deprotonation, N-t order/disorder, free/interfacial enzyme, and extending to specific substrate binding and GS/TS formation. Experimental data for the pH dependence of these equilibria are limited. The N-t pK_a of porcine pancreatic PLA_2 free in solution is about 8.3 [14], and the pH dependence of the interaction between PLA_2 and micelles gives a pK_a of a similar value [4]. Therefore the free solution N-t pK_a could be determining interfacial binding, and the N-t pK_a in the interfacial complex will be around 8–9 or larger. If this interfacial pK_a (in the GS) is around 9, then adding the unfavourable N-

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PII S0014-5793(97)00119-1

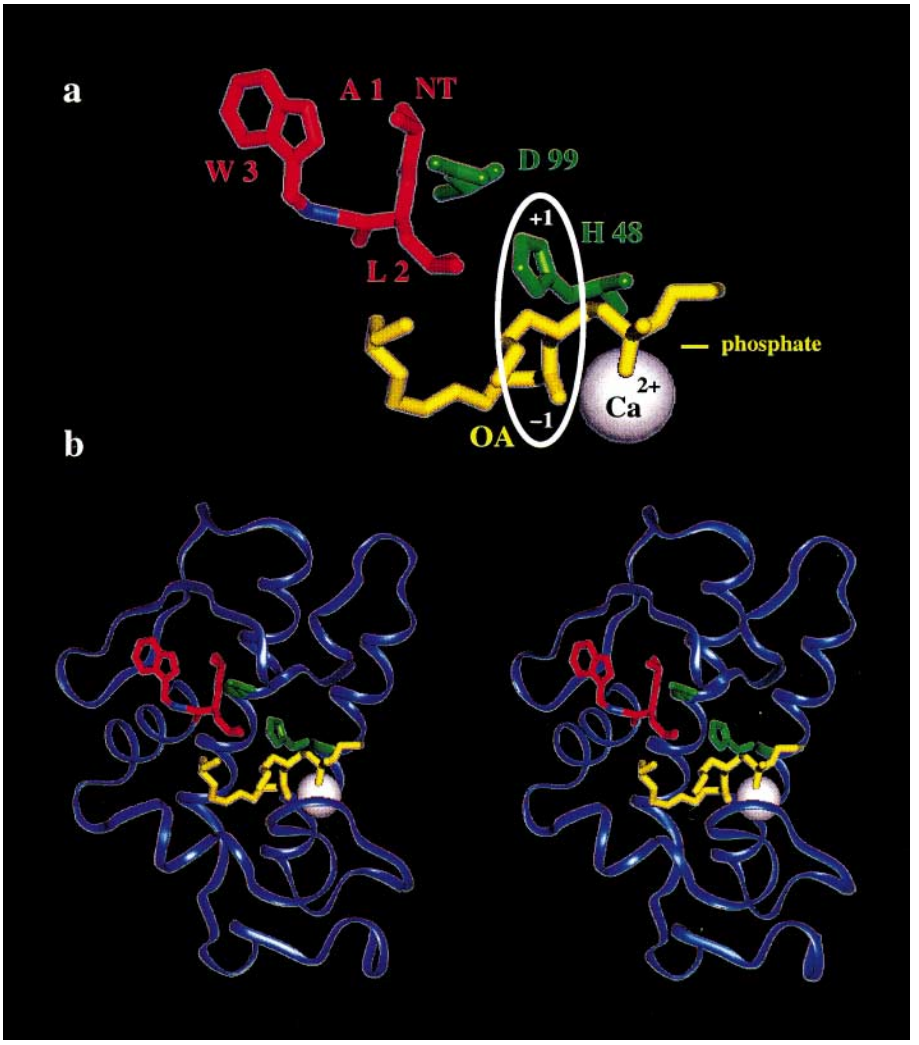

Fig. 1. Model for the dipolar TS charge. (a) Detail of the relevant regions, with inhibitor in yellow (including modelled oxyanion), essential calcium (white), D99 and H48$^+$ (green), and those N-t regions which are removed when modelling N-t disorder (red). (b) The same groups are shown in a stereo view of the whole enzyme.

t$^+$-H48$^+$ interaction, which develops in the TS, a pK_a of around 7 would be expected. The influence of this potential TS pK_a is worth considering in view of our incomplete understanding of the factors that contribute to the pH dependence of PLA_2 activity [9]. In this case, for an enzyme engaged in the catalytic cycle at pH 8, the ordered N-t (left panel of Fig. 2A) would tend to deprotonate during the lifetime of the TS. Such deprotonation would be more likely to favour N-t disorder in the free enzyme, (which may therefore tend towards the right-hand panel of Fig. 2A), than in the interfacially bound enzyme. It is known that N-t order mediates interfacial binding [6], so that the N-t will only become disordered if the enzyme is released from the interface within the lifetime of the TS. Whilst the N-t protonation/deprotonation is likely to be fast, interfacial binding/release may be relatively slow in the context of catalytic turnover [15], so that the centre panel of Fig. 2A could represent interfacially bound PLA_2, if the N-t pK_a in the interfacial TS complex is less than the pK_a of 8–9 observed to mediate interfacial binding [4].

Based on this assumption about the N-t pK_a in the interfacial TS complex, the model predicts distinct catalytic behaviours versus monomeric and aggregated substrates in the pH range between the N-t TS pK_a and 8–9. A contribution to IA of 9.3 kJ/mol in terms of TS stabilisation, equivalent to a rate enhancement of about 40 at 300 K, is predicted. Since the direct and unfavourable N-t$^+$-H48$^+$ interaction is predicted to be alleviated for both interfacially bound and non-bound enzymes within this pH range, IA arises from the charge burial term which is retained for interfacially bound PLA_2. This effect should manifest as an increase in interfacial activity around the N-t TS pK_a. A pK_a of around 7 is observed in studies of PLA_2 activity, and an association with the N-t has been suggested previously [9], but without the potential link to IA provided by the calculations of Fig. 2A. The model predicts a relatively smooth pH dependence for non-interfacially bound PLA_2 activity since the two $\Delta\Delta G_{es}^{TS}$ terms of Fig. 2A approximately cancel. Experiment gives a small increase in this activity as pH increases [16] suggesting that, within the framework of the model, the two $\Delta\Delta G_{es}^{TS}$ terms do not precisely cancel. The important prediction of this approximate model is that an N-t mediated contribution to IA would give rise to a significantly greater pH dependence of activity

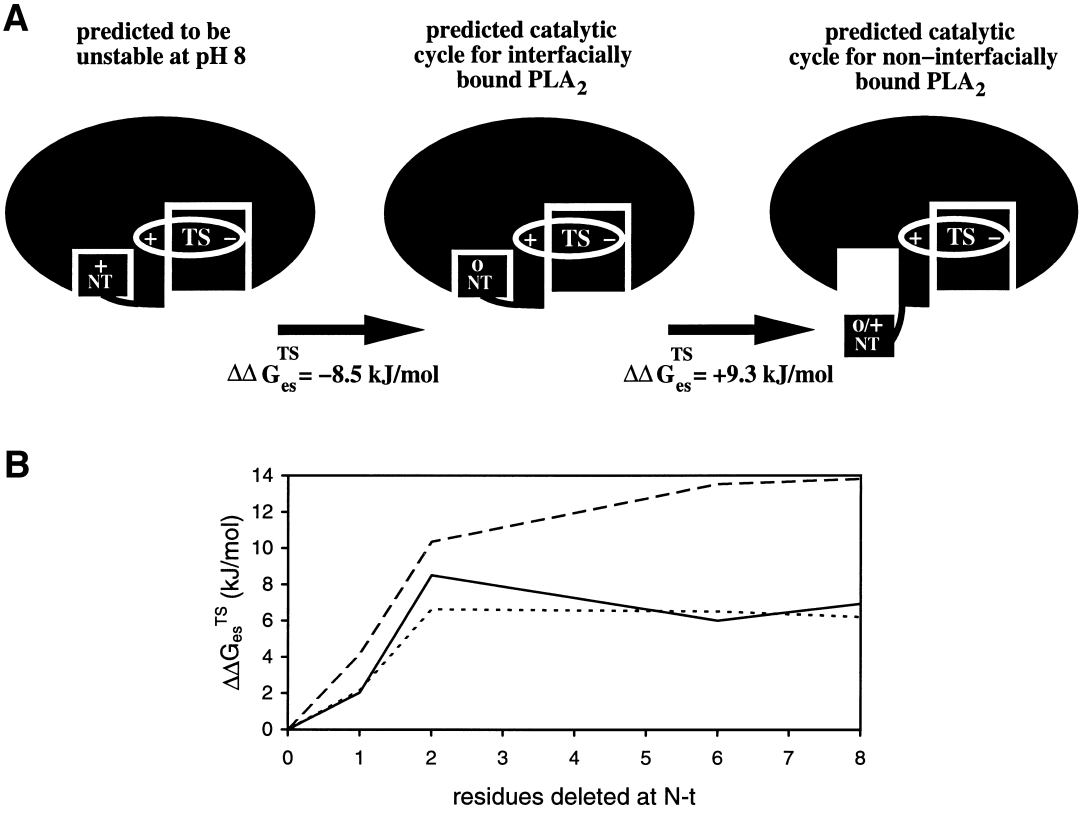

Fig. 2. (A) Calculated $\Delta\Delta G_{es}^{TS}$ upon N-t deprotonation and disordering. Titles associate each schematic panel with a predicted form of PLA_2 during the lifetime of the TS dipole. Protonation of the disordered N-t in the right-hand panel is predicted to give insignificant $\Delta\Delta G_{es}^{TS}$ since the N-t^+-$H48^+$ interaction will be damped by intervening solvent. (B) Comparison of experiment and theory for N-t deletions. Experiment [18] is drawn with a solid line and calculated values of $\Delta\Delta G_{es}^{TS}$ are shown for the charge burial term (long dashes) and for the protonated N-t term (short dashes).

with aggregated substrate than with monomeric substrate. The N-t of the class III bee venom PLA_2 is entirely different to those of class I and II PLA_2s [2], and this enzyme does not exhibit IA [17], consistent with the current hypothesis.

Interfacial activities have been reported for semi-synthetic PLA_2s with a range of N-t deletions [18]. For comparison to calculation these activities have been converted to equivalent TS stabilisations (relative to wild-type), thus providing an interpretation solely in terms of k_{cat}, for a process that could derive from a combination of k_{cat} and K_m effects. Both calculated $\Delta\Delta G_{es}^{TS}$ terms of Fig. 2A are shown in Fig. 2B. If the various amino-termini are deprotonated during interfacial catalysis, the charge burial term (with neutral N-t) is likely to be more relevant. Two interesting correlations between experiment and theory are apparent. First, amino acids 1 and 2 are the most important, with further deletions having relatively small effects. Second, residue 2 is as important as the wild-type amino-terminal residue itself. These correlations support the predicted contribution of the N-t to interfacial activity through k_{cat} and TS stabilisation.

A large part of the predicted IA term due to charge burial arises from enhanced $D99^-$-$H48^+$ interaction with an ordered N-t. Aggregated and monomeric substrate activities have been reported for the D99N mutant of porcine pancreatic PLA_2 [19]. Activity is reduced 25-fold on aggregated substrate, but by only 65% on monomeric substrate, consistent with an enhanced TS stabilisation due to $D99^-$ in the interfacial complex. For the equivalent mutation in bovine pancreatic PLA_2 [20], the apparent micellar k_{cat} decreases 210-fold, and the monomeric k_{cat} 13-fold. The unexpected increase of the activity-related pK_a [20], which the current model associates with N-t deprotonation in the TS, may be due to increased solvent exposure of the N-t in the mutant enzyme [20]. If the apparent micellar k_{cat} reflects TS stabilisation, then the substantially larger drop in interfacial activity for the mutant would again correlate with the predicted importance of $D99^-$ in IA. It should be noted that an alternative hypothesis, based on the complex kinetics at interfaces with variable substrate and neutral diluent composition [15], interprets PLA_2 IA in terms of enhanced substrate binding to the active site upon interfacial binding, and is therefore of K_m type rather than k_{cat} type [21]. The N-t mediated k_{cat} hypothesis provides a simple molecular model for a large part of IA in PLA_2s, and a clear-cut prediction is the existence of a transiently deprotonated N-t for interfacially active enzyme. This model is based on an assumption about the size of the N-t pK_a in the interfacial complex. Correlations with biochemical data for N-t deletion and D99 mutation, and with the pH dependence of activity, support the notion that N-t charge and conformation play important roles in PLA_2 catalysis through electrostatic interactions. The detailed bases of the modelling, in particular the relevant pK_a values, will require further experimental investigation.

Interface charge exerts an additional control on PLA_2 activity, with increased lipolysis reported for the porcine pancreatic enzyme with negatively charged relative to neutral sub-

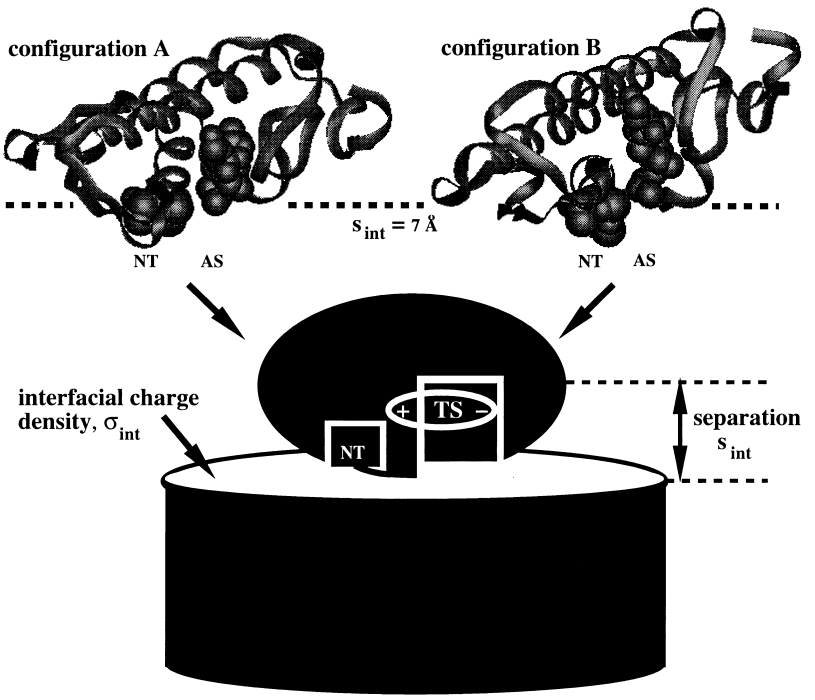

Fig. 3. The 2 modelled interfacial configurations (A,B) of PLA_2 are shown, each with N-t and active site (AS) marked, together with a schematic illustration of the system used to calculate interfacial effects, and the location of the closest interfacial approach (s_{int} = 7 Å).

strates [22,23]. These activity changes have been interpreted in terms of both K_m and k_{cat} effects. The interfacial binding regions of many PLA_2s are positively charged [24], so that a negatively charged interface would promote binding. Fig. 3 shows a model system designed to test whether interfacial charge could alter TS stability and k_{cat}. Changes in ΔG_{es}^{TS} are studied as enzyme in 2 different orientations is brought to a separation of s_{int} with a cylindrical disk of low dielectric and surface charge density σ_{int}. Both orientations present the active site and the N-t to the interface. In configuration A the calcium binding loop is closer to the interface, whereas in configuration B a larger extent of the N-t α-helix has been oriented towards the interface.

Fig. 4 shows $\Delta\Delta G_{es}^{TS}$ contributions from σ_{int}, from the N-t positive charge, and from other protein charges (and the essential calcium), plotted against s_{int}. For both configurations the $\Delta\Delta G_{es}^{TS}$ term due to protein charges (and the essential calcium) other than the N-t is small, whilst that due to the N-t charge increases moderately due to the low dielectric (solvent excluded) environment. The term due to a negative σ_{int}, representative of an anionic phospholipid, is more substantial and variable. Experiment has not yet provided a picture of the interfacial complex, so that detailed enzyme orientation and separation are unknown. At s_{int} = 10 Å the sidechain of W3 is within the interfacial slab and burial of charged amino acid sidechains is avoided so that separations around this value, which approximates to the loose complex in a reported molecular dynamics study [25], may be representative. Fig. 4 shows that $\Delta\Delta G_{es}^{TS}$ due to a negative surface charge can be significant (−15 kJ/mol for configuration B at s_{int} = 10 Å), and is highly dependent on orientation (compared to 0 kJ/mol for configuration A at s_{int} = 10 Å). The variability arises from an almost parallel orientation of the TS dipole to the interface. Alterations in PLA_2 configuration at the interface would therefore permit interactions between σ_{int} and either $H48^+$ or the oxyanion to predominate, giving a mechanism linking detailed interfacial binding and σ_{int} mediated k_{cat} modification. Whereas porcine pancreatic PLA_2 may bind in a configuration giving favourable $\Delta\Delta G_{es}^{TS}$ with negative σ_{int}, those snake venom enzymes for which activity is decreased at a negatively charged interface [22] may bind with dominant interactions between σ_{int} and the oxyanion. Additionally, the N-t pK_a may vary in the presence of interfacial charge. Such variation would, based on the current model, alter the pH dependence of activity.

In conclusion, electrostatic calculations indicate that k_{cat} for PLA_2 will depend significantly on N-t charge and conformation, and on interface charge and detailed interfacial complex structure. Consideration of N-t charge interactions with the TS, together with an assumption about the size of the N-t pK_a in the interfacial complex, lead to the suggestion that transient deprotonation of an ordered N-t in the interfacial complex could contribute significantly to IA. It is proposed that the transient deprotonation could occur on a faster time scale than interfacial release. It is possible that in cases of relatively weak interfacial binding, or of a rapid release step, the time scales for the proposed deprotonation and for release could overlap in such a manner as to further complicate the separation of k_{cat} and K_m effects in interfacial catalysis. The predicted dependence of k_{cat} on σ_{int} is linked to the detailed structure of the interfacial complex, providing a mechanism for species variation of activity. Such dependence could also complicate kinetic measurements made with interfaces of varied neutral diluent and anionic substrate composition. The hypothesis is in agreement with several lines of experimental evidence, notably the division of IA in PLA_2s with and without the implicated N-t configuration, deletion analysis at the N-t, mutation analysis of D99, and the dependence of activity on interfacial charge. Whereas a study of global charge fields in PLA_2s argued against a simple electrostatic correlation be-

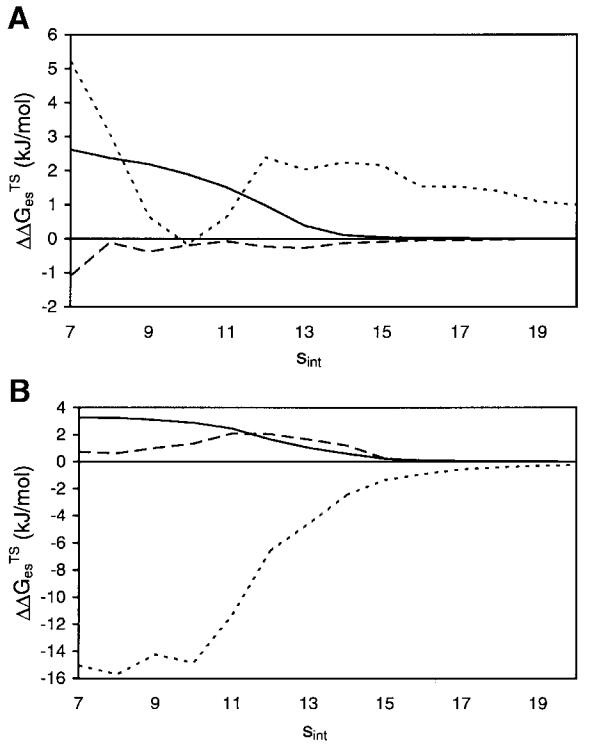

Fig. 4. Calculated $\Delta\Delta G_{es}^{TS}$ (relative to PLA_2 with no interface) is plotted against s_{int} for configuration A (A), and configuration B (B). In each panel the solid line gives the contribution from the charge of a protonated and ordered N-t, the long dashes denote that from other enzyme charges, whilst the short dashes represent the σ_{int} contribution.

tween the N-t and IA [24], the current model incorporates the TS and finds such a correlation. The prediction of a transiently deprotonated N-t during interfacial catalysis should allow experimental investigation of the hypothesis.

Acknowledgements: This work was supported by the UK BBSRC. The author thanks the referees for their comments.

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