Eur Biophys J (1998) 28: 67–73

ARTICLE

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Numerical simulation of aldolase tetramer stability

Received: 16 February 1998 / Revised version: 8 April 1998 / Accepted: 19 April 1998

Abstract A theoretical study of aldolase tetramer stability, conducted by finite difference Poisson-Boltzmann (FDPB) and modified Tanford-Kirkwood (MTK) techniques using the atomic coordinates of human aldolase, is described. A method for calculating the interaction energy between subunits is proposed. An analysis of the contribution of different energy terms to the stability and oligomeric equilibria (monomer ⇔ dimer ⇔ tetramer) of aldolase is made. It is shown that the loss of solvation energy and electrostatic interactions at very high and low pH-s destabilise the oligomers. These energy terms are compensated over a wide pH range by the stabilization energy due to hydrophobic interactions. It is shown that the aldolase tetramer is energetically more preferable than other oligomers in the pH range from 5 to 11. Subunit-subunit interactions within the tetramer suggest one dimeric form to be the most stable of the possible sub-parts. For this reason the tetramer can be thought of as a "dimer of dimers". A comparison between our theoretical results and available experimental data shows that the dissociation of the aldolase tetramer below pH 3–4 cooperatively leads to acid denaturation. A second dissociation is predicted to occur at high pH (>12) in addition to the well known acidic dissociation. The analysis suggests that a mutation of His20 or Arg257 to a neutral residue could decrease the pH of the acidic dissociation by approximately 1 pH unit.

Key words Protein stability · Electrostatic interactions · Aldolase

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Abbreviations *FDPB* Finite difference Poisson-Boltzmann · *MTK* Modified Tanford-Kirkwood · *vdW* van der Waals · *L* Oligomer · *M* Monomer · *D* Dimer (D_{ab}, D_{ac} and D_{ad}) · *T* Trimer and Q, tetramer or Quaternary structure · *SAS* Solvent accessible surface · *SA* Static accessibilities

Introduction

Most enzymes are oligomeric molecules. The association of the subunits is a necessary condition for biological activity of most of these proteins. In some instances the active site is located in the space between subunits; in other instances the specific interactions between subunits facilitate the biological function of proteins (Cantor and Schimel 1980).

A typical example of such an enzyme is aldolase. The aldolase molecule has four identical subunits (here named a, b, c, and d) packed in a tetrahedral-like tetramer (Sygusch et al. 1987; Blom and Sygusch 1997). Because only the aldolase tetramers are biologically active, while the monomers are not (Engelhart et al. 1976; Morris and Tolan 1994), many experiments have been carried out in order to measure the stability of the tetramer structure. The quaternary structure has been studied by site-directed mutagenesis at the subunit interface (Beernik and Tolan 1994, 1996). The experiments have shown that the four-subunit (Q) structure of the enzyme is not dissociated to trimeric (T) forms but into monomers (M) by guanidine hydrochloride (Kawahara and Tanford 1966) or into a dimer(D)-monomer mixture by acid (Ohga et al. 1984). The acidic dissociation is accomplished by pH-denaturation (Engelhart et al. 1976). The available experimental data can be compared with the results of our numerical approach.

There is a well established physical analogy between subunit association within a quaternary structure and protein crystal lattice formation. One of the forces responsible for the association of subunits within oligomers, as well as molecules within protein crystals, is the electrostatic force. The effect on protein crystal stability has been simulated

numerically (Takahashi et al. 1993; Alexov and Atanasov 1994) for various proteins. It was shown that electrostatic interactions play an essential role in protein assembly, thus it is important to study the effect of pH on the oligomer stability.

The principal aim of this work is to investigate numerically the stability of the effector-free aldolase tetramer (Q) and its sub-parts (M, D and T) as a function of pH. Calculations are carried out for each of the oligomeric forms at the atomic level and this permits us to find the macroscopic parameters by which the oligomers are stabilized and indicate the residues responsible for the subunit association. Our results are then compared with the available experimental data of pH-dissociation of aldolase (Ohga et al. 1984).

Methods

a) Data files

Atomic coordinates of the aldolase subunit are taken from the Protein Data Bank (Bernstein et al. 1977), entry 1ald (Gamblin et al. 1990). Our numerical analysis is based on these fixed coordinates of human substrate/product-free aldolase. The structure of the human muscle aldolase is highly homologous with that of rabbit (Sygusch et al. 1987). The tetramer structure is reconstructed on the basis of atomic coordinates of one subunit using transformation functions of the 222-fold crystal space group.

b) Calculation of the average charge of the titratable groups as a function of pH

In this work we evaluate and compare the free energies of the oligomeric (Q, T and D) and monomeric forms of aldolase at different pH-s. For this purpose we first calculate the free electrostatic energy using the average charges of the ionizable residues at each pH. Then we evaluate the pH-independent terms and add them to the free electrostatic energy.

In order to calculate the average charges of titratable groups (q_i) at a given pH, we follow the well developed methods described elsewhere (Yang et al. 1993; Bashford and Karplus 1990). We assume that the pK_a's of the free residues in solution (pK_{mod}) are modified when these residues are a part of the protein due to three main factors. First, the change in solvation energy on transfer of a charge from the highly polar water environment to a protein with (usually) restricted solvent accessibility (Born energy) causes the ΔpK_{Born} shift. Second is the so called ΔpK_{back} shift, arising from the interaction of titratable residues and the permanent partial atomic charges. Third is the free energy shift (ΔpK^{tit}) that corresponds to the interaction energy between titratable residues within the protein.

These energies are calculated using the finite-difference method (Glison et al. 1987), and by solving the Poisson-Boltzmann equation in each cube of the space grid with $65\times65\times65$ nodes. Dielectric constants for protein and solution are chosen to be 4 and 80, respectively. The partial atomic charges are based on the AMBER force-field (Weiner et al. 1984). The solvent accessible surface (SAS) and the static accessibilities (SA) are determined following Lee and Richards (1971). Calculations were made at ionic strength $I=0.1$.

The aldolase tetramer consists of 440 titratable residues. Such a number of ionizable groups is too large for the statistical average method (Bashford and Karplus 1990). A reduced site approximation is usually used for proteins containing 50–80 titratable groups (Bashford and Karplus 1991). In our case, only the main field approximation and a MTK approach (Karshikoff et al. 1989) are realistic methods for obtaining the average charges. We calculate these charges by the FDPB method following the procedure described by Karshikoff (1995). Applying the MTK approach we use a semiempirical potential function to evaluate pair interactions (Spassov et al. 1989).

By definition the average charges are $q_i(pH)=q_0+\alpha_i$, where $q_0=-1$ for acidic groups and $q_0=0$ for basic groups. At a given pH, the degree of protonation, α_i, of the ith titratable group, can be calculated by the Linderstrøm-Lang relationship:

$$\log\left[\alpha_i/(1-\alpha_i)\right]=pK_i-pH. \tag{1}$$

The pK_a value of the ith ionizable group in a protein molecule is pH-dependent, $pK_i(pH)$, and can be determined

$$pK_i(pH)=pK_{i,int}+\Delta pK_{i,tit}(pH) \tag{2}$$

where $pK_{i,int}$ is the intrinsic pK_a value and $\Delta pK_{i,tit}(pH)$ is the ΔpK_a shift due to the electrostatic interactions between the ith and all other titratable groups. The term $\Delta pK_{i,tit}$ is calculated by:

$$\Delta pK_{i,tit}=-\frac{1}{2.303\,RT}\sum_{\substack{k=1\\k\neq i}}^{N} q_k(pH)\,\varphi_{ik} \tag{3}$$

where φ_{ik} is the potential generated by the ith charge in the location of the kth charge. The φ_{ik} values are obtained by FDPB and MTK methods, respectively. The first correction of the pK_i value is calculated by Eq. (1). The values of pK_i are finally obtained by an iterative procedure (Karshikov et al. 1989).

The intrinsic pH-independent $pK_{i,int}$ of the titratable group, as described above, is defined as:

$$pK_{i,int}=pK_{i,mod}+(\Delta pK_{i,Born}+\Delta pK_{i,back}). \tag{4}$$

We calculate average charges and pK_a's of the ionizable groups as a function of pH in the monomer (q_i^M and pK_i^M) as well as in the oligomers (q_i^L and pK_i^L): three possible dimers (D_{ab}, D_{ac}, D_{ad}), a trimer and the tetramer.

c) Free energy calculations

We would like to note that we calculate the total free energy that stabilizes oligomeric structures and the methods

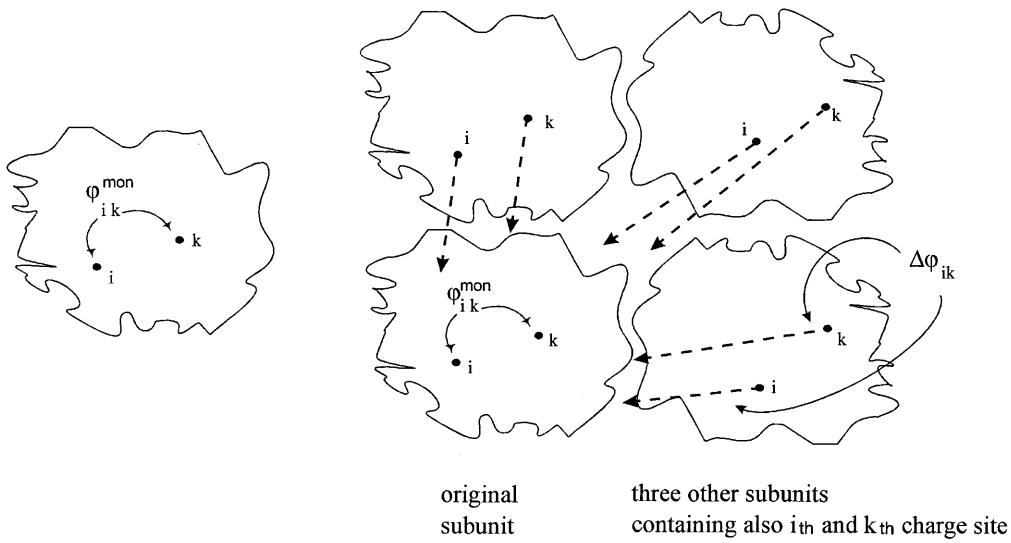

Fig. 1 Schematic representation of the isolated subunit and the tetramer structure. When the subunit is a part of the tetramer structure, then the potential φ_{ik}^{M} is perturbed to be the potential generated by the same charged sites belonging to the neighbouring subunits

used do not allow separation of the individual enthalpy and entropy contributions. The free energy represents the interactions between subunits within a given oligomer and contains the main terms described above, the pH-independent "neutral" term $\Delta\Delta G^{neut}$ and the pH-dependent "titration" term $\Delta\Delta G^{tit}$. We consider every monomer as a rigid body, assuming that it does not change its structure in the pH range studied and consequently its "self energy". Following this assumption, we neglect the change of self energy of the monomer upon association. This is an important aspect of our study, and one of the main differences between our methodology and that currently employed in studying protein stability (Yang and Honig 1993).

The stability of the aldolase oligomers is analysed on the basis of the free energy difference between the energy of the subunit within the oligomer ΔG^{L} (pH) and the energy of an isolated one, ΔG^{M} (pH):

$$\Delta\Delta G\ (\mathrm{pH}) = \Delta G^{L}\ (\mathrm{pH}) - \Delta G^{M}\ (\mathrm{pH}). \qquad (5)$$

When $\Delta\Delta G$ (pH) has a negative value, the free energy of the subunit within the oligomer is smaller than that of the isolated subunit. In this case, the negative increment of the free energy corresponds to the force of attraction between subunits within the oligomer. In the other case, when $\Delta\Delta G$ (pH) has a positive value, the oligomer structure tends to dissociate into monomers.

The $\Delta\Delta G^{neut}$ term does not depend on titration of the ionizable groups. It arises from the lack of hydrophobic surface when the subunits associate into an oligomeric structure ($\Delta\Delta G^{hydr}$); from van der Waals contacts between subunits within the oligomer under consideration ($\Delta\Delta G^{vdW}$), as well as from electrostatic interactions between permanent atomic partial charges (presented as dipoles, $\Delta\Delta G^{dip}$) belonging to neighbouring subunits and from the Born energy difference ($\Delta\Delta G^{Born}$).

The $\Delta\Delta G^{hydr}$ term is estimated by the loss of accessible surface when the subunit becomes part of an oligomeric structure. The buried surface reduces the hydrophobic interaction by 25 cal/mol Å^2 (Chothia, 1974). The $\Delta\Delta G^{vdW}$ term is estimated by the number of vdW contacts between subunits within the oligomer. It is assumed that two atoms have vdW contact if the distance between their centres is approximately 3 Å. Following Moore (1962), every vdW contact contributes to the $\Delta\Delta G^{vdW}$ a value of approximately 45 cal/mol.

The $\Delta\Delta G^{dip}$ accounts for the difference in polar interaction energies between monomer and tetramer. The $\Delta\Delta G^{dip}$ energy is calculated by putting partial charges on all atoms within the protein except for charges of titratable groups. It explicitly includes hydrogen bonding energy between subunits. The partial charge of each hydrogen belonging to a polar group is multiplied by the potential of the dipoles of the neighbouring molecule, including the potential generated by the eventual oxygen acceptor. The last term $\Delta\Delta G^{Born}$ describes the change in the Born free energy of a given residue when the subunit is associated. The dipole interaction shift (as $\Delta pK_{i,\,back}$.) and the Born free energy shift (as $\Delta pK_{Born,\,i}$) are taken into account in the calculation of the pK_a's of the ionizable groups (see previous paragraph).

The pH-dependent energy difference $\Delta\Delta G^{tit}$(pH) is calculated by both FDPB and MTK methods using already obtained charges of the titratable groups $q_i^{L,M}$(pH), $i = 1, 2, \ldots, N$ (N is the number of titratable residues).

Using the FDPB method the "titratable term" of the free energy difference $\Delta\Delta G^{tit}$ (pH) between an isolated subunit and the tetramer was calculated using:

$$\Delta\Delta G^{tit}(\mathrm{pH}) = \frac{1}{2}\sum_{i=1}^{N}\sum_{k=1}^{N} q_i^{Q}\ (\mathrm{pH})\ q_k^{Q}\ (\mathrm{pH})\ \Delta\varphi_{i,k} \qquad (6)$$

where φ_{ik} is determined as:

$$\Delta\varphi_{i,k} = \varphi_{i,k}^{Q} - \varphi_{i,k}^{M}. \qquad (7)$$

Using Eqns (6) and (7), we cancel out the "self energy" of the subunit, taking into account only the additional potential $\Delta\varphi_{ik}$ generated by the *i*th and *k*th charges on the other three subunits within tetramer (Fig. 1).

Using the MTK method the calculations of the pH-dependent free energy difference $\Delta\Delta G^{tit}$ between the isolated

Table 1 The neutral free energy contributions ($\Delta\Delta G^{hydr}$, $\Delta\Delta G^{vdW}$, $\Delta\Delta G^{neut}$) estimated by the solvent accessible surface (SAS) and the number of vdW contacts at oligomerization (N_{vdW}) in different oligomeric variants of aldolase. All data are presented for one subunit

Oligomer	SAS (Å^2)	N_{vdW}	Free energy differences (kcal/mol)		
			$\Delta\Delta G^{hydr}$	$\Delta\Delta G^{vdW}$	$\Delta\Delta G^{neut}$
Dab	106.0	0	–2.7	0.0	–2.7
Dac	899.0	203	–22.5	–9.5	–32.0
Dad	9.5	0	–0.2	0.0	–0.2
Tabc	1005.0	203	–25.1	–9.5	–34.6
Q	1014.0	203	–25.4	–9.5	–34.9

subunits and all possible oligomeric forms were carried out. We would like to emphasize that our variant to the MTK method includes an evaluation of all terms described in Eqns (2)–(4) (Villoutreix et al. 1994).

Results

We note that all the results are shown as per subunit. The hydrophobic part of the pH-independent free energy ($\Delta\Delta G^{hydr}+\Delta\Delta G^{vdW}$) contributing to the stability of the oligomeric forms of aldolase was determined by evaluation of the buried area on oligomerization and the number of vdW contacts on oligomerization (see columns 2–3 in Table 1). Following the methodology described above, the values of $\Delta\Delta G^{hydr}$ and $\Delta\Delta G^{vdW}$ of the different oligomeric forms are calculated (columns 4–5 in Table 1).

The electrostatic free energy difference of a tetramer and an isolated subunit $\Delta\Delta G^{tit}$ (pH) obtained by the FDPB method is shown in Fig. 2, curve 1. It is seen that the electrostatic energy decreases from 37 kcal/mol at pH 1 to 13 kcal/mol in the pH range 8–9.

The pH dependence of the dissociation free energy of the aldolase tetramer $\Delta\Delta G$ (pH) based on the FDPB technique is shown in Fig. 2, curve 2. It is obtained by summing the pH-dependent electrostatic $\Delta\Delta G^{tit}$ (Fig. 2, curve 1), and pH-independent contributions of $\Delta\Delta G^{neut}$ (see Table 1). The value of the dissociation free energy of the tetramer slowly decreases from 3 kcal/mol at pH 1 to a few kcal/mol in the pH range 2–4. Above pH 4.5 the $\Delta\Delta G$ (pH) values decrease to –23 kcal/mol at pH 8.5.

Using the MTK method the pH-dependent stability of all oligomeric forms is considered. It should be noted that the experimentally determined isoelectric point of the tetrameric aldolase is in the pH range 8.1–8.3 (Kent and Lebherz 1984). The calculated titration curves show protein net charge Z = 0 at pH 8.3 and 8.7 for the tetramer and the monomer, respectively.

The electrostatic free energy differences calculated by the MTK method of the dimeric and trimeric forms ($\Delta\Delta G^{tit}$) are shown in Fig. 3. The electrostatic stability of both Dab and Dad dimers (curves 1 and 3) are very close to that of the monomer. The values of $\Delta\Delta G^{tit}$ (pH) have a

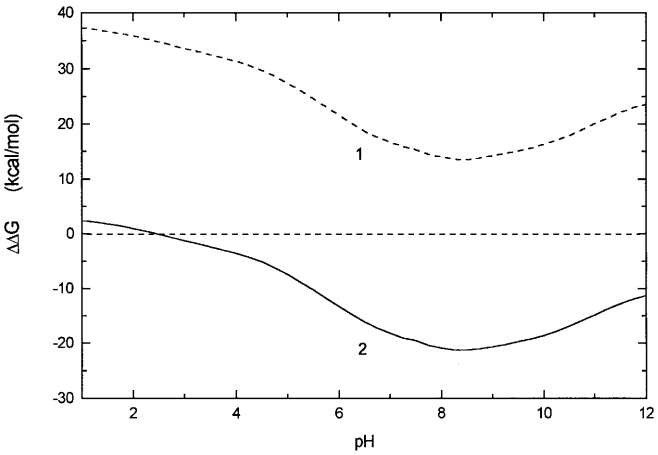

Fig. 2 *1* The electrostatic free energy of interaction between subunits within the tetramer ($\Delta\Delta G^{tit}$) calculated by the FDPB technique; *2* The dissociation/association free energy ($\Delta\Delta G$) of the aldolase tetramer by means of monomers as a function of pH, calculated by the FDPB technique

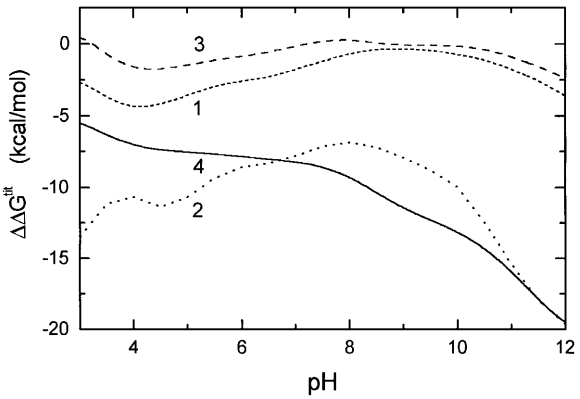

Fig. 3 The electrostatic free energy of interaction between subunits ($\Delta\Delta G^{tit}$) calculated by the MTK method. *1* within the dimer D_{ab}; *2* within the dimer D_{ac}; *3* within the dimer D_{ad}; *4* within the trimer

magnitude of ±2 kcal/mol in the pH range 5–12 and this shows slight intersubunit electrostatic interactions in these dimers. The electrostatic stability of the trimeric form is increased by 6–8 kcal/mol in the pH range 4–10 (curve 4). The parallel pH-dependent changes in the trimer and dimers D_{ab} and D_{ad} suggest a coupling of some charges from D_{ab} and D_{ad} into the trimer. The pH-dependent electrostatic stability of D_{ac} is presented by curve 2 (Fig. 3). The larger electrostatic stability of D_{ac}, as well as the close electrostatic energy difference of D_{ac} and the trimer suggest the formation of "internal dimers" $D_{ac} \equiv D_{bd}$. The presence of a large number of subunit-subunit contacts and resultant changes in the SAS of ionizable groups increases the total stability of $D_{ac}=D_{bd}$ dimer in comparison with other pairs D_{ab} and D_{ad} (see Table 1).

The tetramer electrostatic pH-dependent stability calculated by the MTK method is shown in Fig. 4A. Curves 1 and 2 represent the electrostatic free energy difference of the tetramer and the monomer and the most stable dimer

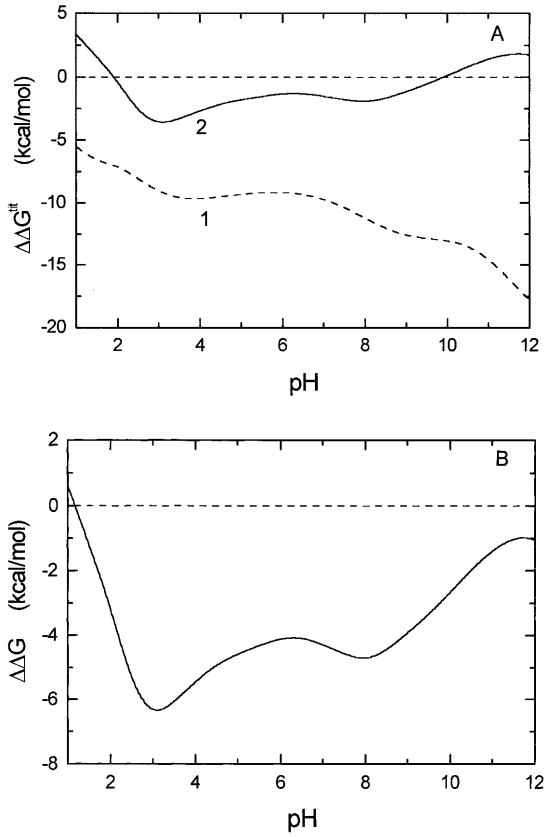

Fig. 4 **A** The electrostatic free energy difference ($\Delta\Delta G^{tit}$) calculated by the MTK method. *1* obtained by comparison of tetramer and monomer; *2* obtained by a comparison of tetramer and dimer D_{ac}. **B** The dissociation/association free energy ($\Delta\Delta G$) of the aldolase tetramer by means of dimers as a function of pH, calculated by the MTK method

D_{ac}, respectively. In Fig. 4B is shown the total free energy difference between the tetramer and the dimer D_{ac} calculated using the MTK method. The stability curve is obtained by summing the electrostatic term ΔG^{tit} (pH) and the pH-independent hydrophobic terms, $\Delta\Delta G^{hydr}$ and $\Delta\Delta G^{vdW}$, (see Table 1). The results obtained show the tetramer probably dissociates to dimers D_{ac} in the acidic range below pH 2.5 and in the alkaline range above pH 10.

Figure 5 shows the changes of the static accessibilities (ΔSA) for the titratable groups due to $4M \Leftrightarrow 2D \Leftrightarrow Q$ transitions. It is seen that some groups become deeply buried upon association. There is one titratable group changing its accessibility from the dimer D_{ac} to the tetramer–His156. Others ionizable groups lose the accessibilities upon a dimerization in D_{ac}.

Discussion

The analysis performed above shows that the aldolase tetramer is stable in a wide pH range. That is due to forces of different origin. The pH-independent energy $\Delta\Delta G^{neut}$, which stabilizes the tetramer structure, is always negative. The pH-dependent term $\Delta\Delta G^{tit}$ (pH), the electrostatic energy difference between the tetramer and monomer structures, has minimal values at approximately pH 8–9 (Fig. 4A, curves 1). At pH near the calculated isoelectric point 8.2 for the tetramer and 8.9 for a monomer, the net charge of the subunits is zero, and they interact with each other because of their multipole moments. It is well known that the energy of multipole interaction is lower than that of single-charge interaction. In the opposite case, when the pH is above or below that of the isoelectric point, the subunits have a net charge. As a result, the electrostatic energy of interactions between subunits increases with increasing/decreasing pH with respect to the isoelectric point. This process continues up to the pH limit at which all basic groups are completely ionized (at very low pH) or at which all acidic groups bear full charge (at very high pH). Below pH_{low} and above pH_{high} the electrostatic energy of the interaction between subunits remains constant because the subunits have already reached their maximal net charge.

The result from the FDPB method suggests the tetramer structure is stable in the pH range 5-13 (Fig. 2, curve 2). Curve 2 monotonically decreases as pH increases from 1 to 8.5 and then begins to increase at pH > 9. However in the pH range 2.5–4.0 the curve shape is little changed. In this pH range, $\Delta\Delta G$ (pH) decreases more slowly than in other pH intervals. $\Delta\Delta G$ is zero at pH 2.7. This can be understood as a pH range of dissociation of the tetrameric form. Comparing this numerical result with the experiments directed at studying the acidic dissociation of aldolase (Engelhart et al. 1976; Kent and Lebherz 1984), we find very good agreement.

The results of electrostatic stability of the tetramer in comparison with the monomer, obtained by the MTK method (Fig. 4A, curve 1), are not reasonable. The cecrease of $\Delta\Delta G^{tit}$ above pH 10 shows that the fixed-coordinates model in the alkaline range is not correct. An alkaline denaturation above pH 10 is quite possible. Adding $\Delta\Delta G^{neut}$ to $\Delta\Delta G^{tit}$, calculated by the MTK method, for the $Q \Leftrightarrow M$ transition (Fig. 4A, curve 1) gives as a result negative $\Delta\Delta G$ values. Such a result does not suggest acidic dissociation, which is contrary to the experimental results. On the other hand, the total $\Delta\Delta G$ for the equilibrium $D_{ac} \Leftrightarrow Q$ (Fig. 4B) quite well predicts the acidic dissociation below pH 2. These results suppose that the tetramer probably dissociates first to dimers and then to monomers. This conclusion shows the coincidence of the results obtained by both theoretical methods. Experiments in this pH range observed a mixture of tetramers, monomers and dimers (Ohga et al. 1984). This means that the energy difference between the above mentioned forms in this pH range is not very large. These are the same results (Fig. 2, curve 2 and Fig. 4B) we obtain by our numerical simulations. This is an additional confirmation that the models introduced can correctly predict the stability of the oligomer structure.

In this work we have calculated the energies that stabilize/destabilize the tetrameric form of aldolase. Bearing in mind that only the tetramer is biologically active, we can

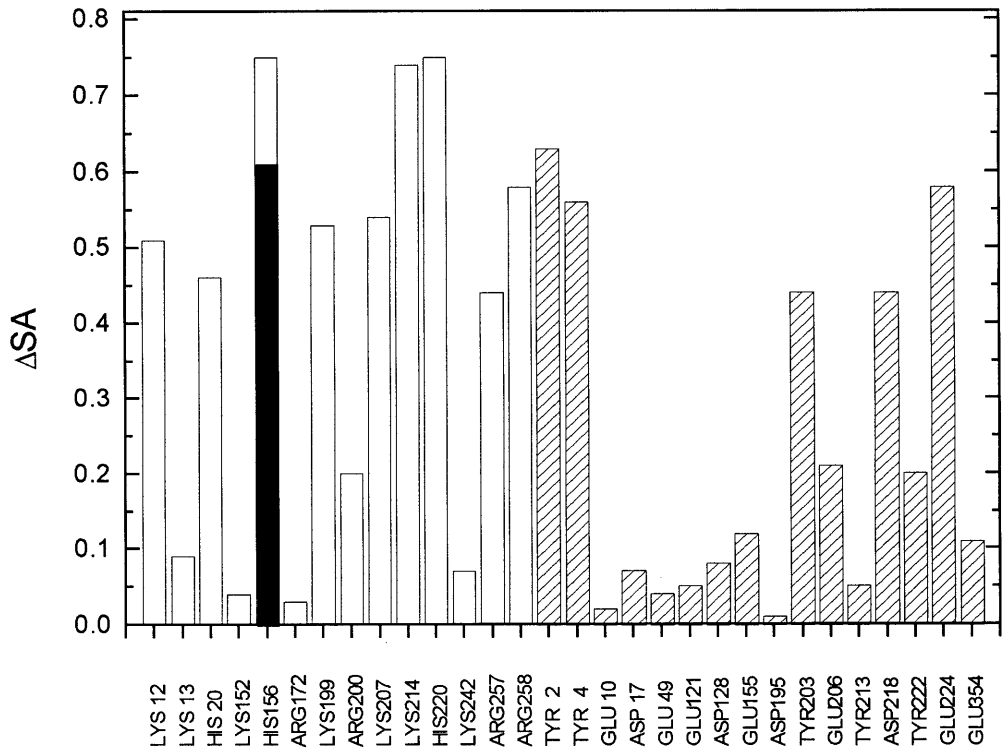

Fig. 5 The changes of static accessibility (ΔSA) for the residues belonging to the buried area in the tetramer (the *black bars*) and in the dimer D_{ac} (the white bars – basic groups; the *shaded bars* – acidic groups)

compare our results (Fig. 4B) with the pH-dependent activity of this enzyme (Morris and Tolan 1994). Again we find good agreement. The minimum of ΔΔG (pH) about pH 9 agrees with the conclusion (Misset et al. 1986) that aldolase is a basic enzyme. Our calculations predict that at high pH (pH > 13) one should expect another basic dissociation of aldolase.

The stability of the aldolase tetramer can be analysed from a macroscopic point of view, as well as on the level of its residues. The quaternary structure of human aldolase presents a different number of contacts (N_{vdW}) and buried static accessible surface (SAS) for each of the four identical subunits (see Table 1). Our results show that about 30% of charged residues change their accessibility when the subunits aggregate into a tetramer. This causes a change of their pK_a value, and some of these residues become neutral in a wide pH range. Nevertheless, they have a charge in the monomeric state at a physiological pH. The residue that has no charge in the tetramer structure at the pH under consideration, does not contribute to the $\Delta\Delta G^{tit}$ (pH). Hence, we can conclude that site directed mutation of a charged group not involved in a salt bridge to a neutral residue will stabilize tetramer structure. It will decrease both the Born energy and the electrostatic energy difference. This effect can be observed at low pH because the transition tetramer → monomer will occur at lower pH than in the case of the wild-type aldolase by approximately 1 pH unit. There are four salt bridges inside the monomer with changing accessibility in D_{ac}: Lys13/Asp17, Lys214/Asp218, R258/Glu206, Lys207/Tyr203. Although they are internal, they influence the tetramer stability by the Born energy change. The most important are the intersubunit salt bridges. We found such at the contacts between subunits *a* and *c*, as well *b* and *d*: Lys12/Glu206 and Arg258/Tyr22. We should note the special role of Glu206 and Arg258, which are involved both in the internal and intersubunit salt bridges. The mutations of these groups to neutral residues should decrease the tetramer stability. The residues His20 and Arg257 from subunits *a* and *c* are very close. Thus we can suppose their mutation to a neutral residue will cause an acidic stabilization of the tetramer structure.

All groups mentioned above are responsible for the pH-dependence of the stability of the dimer D_{ac}. The fact that the double mutant Q125D/E224A was found as a monomer (Beernik and Tolan 1996) can be explained by the change of the accessibility of Glu224 inside the dimer D_{ac}. Only His156 is buried as a consequence of formation of the tetramer from both dimers. Its ionization can influence the tetramer stability in respect of pH.

The results of our theoretical study again confirmed that macroscopic methods such as FDPB and MTK can be successfully applied to energy calculation of the protein assembly. The results obtained strongly correlate with available acidic dissociation and predict such in alkaline range. The methods used in this work can be applied to other oligomers.

Acknowledgements This work was partially supported by grant #X-429 from the National Fund "Scientific Researches", Sofia, Bulgaria. The help of Mr. Edward Pass in preparing the manuscript is greatly appreciated.

References

Alexov E, Atanasov B (1994) Analysis of electrostatic interactions in ribonuclease A monoclinic crystal. Biochim Biophys Acta 1206: 55–62

Bashford D, Karplus M (1990) pKa's of the ionizable groups in proteins: Atomic detail from a continuum electrostatic model. Biochemistry 29: 10219–10225

Bashford D, Karplus M (1991) Multiple-site titration curves of proteins: An analysis of exact and approximate methods for their calculation. J Phys Chem 95: 9556–9561

Beernik P, Tolan D (1996) Disruption of the aldolase A tetramer into catalytically active monomers. Proc Nat Acad Sci USA 93: 5374–5379

Bernstein F, Koetzle T, Williams J, Meyer E, Brice M, Rogers J, Kennard O, Shimanouchi T, Tasumi M (1977) The Protein Data Bank: A computer based archival file for macromolecular structures. J Mol Biol 112: 535–542

Blom N, Sygusch J (1997) Product binding and role of the C-terminal region in Class I D-fructose 1,6-bisphosphate aldolase. Nat Struct Biol 4: 36–39

Cantor C, Schimel P (1980) Biophysical Chemistry Part I. The conformation of biological macromolecules. Freeman, San Francisco

Chothia C (1974) Hydrophobic bonding and accessible surface areas in proteins. Nature 248: 338–339

Engelhart M, Rudolph R, Jaenicke R (1976) Equilibrium studies on the refolding and reactivation of rabbit-muscle aldolase after acid dissociation. Eur J Biochem 67: 447–453

Gamblin S, Cooper B, Millar J, Davies G, Littlechild J, Watson H (1990) The crystal structure of human muscle aldolase at 3 A resolution. FEBS Lett 262: 282–296

Gilson M, Sharp K, Honig B (1987) Calculating the electrostatic potential of molecules in solution: Methods and error assessment. J Comp Chem 9: 327–335

Karshikoff A (1995) A simple algorithm for the calculation of multiple site titration. Protein Eng 8: 243–248

Karshikov A, Engh R, Bode W, Atanasov B (1989) Electrostatic interactions in proteins: Calculation of the electrostatic term of free energy and the electrostatic potential field. Eur Biophys J 17: 287–297

Kawahara K, Tanford C (1966) The number of polypeptide chains in rabbit muscle aldolase Biochemistry 5: 1578–1584

Kent C, Lebherz H (1984) Stability of quaternary structure and mode of dissociation of fructosediphosphate aldolase isoenzymes. Biochemistry 23: 5257–5261

Lee B, Richards F (1971) The interaction of protein structures. Estimation of static accessibility. J Mol Biol 55: 379–400

Misset O, Bos O, Opperdoes F (1986) Glycolytic enzymes of trypanosoma brucei simultanneous purification, intraglycosomal concentrations and physical properties. Eur J Biochem 157: 441–453

Moore W (1962) Physical Chemistry, 3d ed. Englewood Cliffs, NY: Prentice-Hall, p. 715

Morris A, Tolan D (1994) Lysine 146 of rabbit muscle aldolase is essential for cleavage and condensation of the C3–C4 bond of fructose 1,6-Bis(phosphate). Biochemistry 33: 12291–12297

Ohga Y, Nakanishi M, Tsuboi M (1984) Kinetics of the conformational change and subunit dissociation of aldolase. Bull Chem Soc Jpn 57: 692–695

Spassov V, Karshikov A, Atanasov B (1989) Electrostatic interactions in proteins. A theoretical analysis of lysozyme ionization. Biochim Biophys Acta 999: 1–6

Sygusch J, Beaudry D, Allaire M (1987) Molecular architecture of rabbit skeletal muscle aldolase at 2.6 A resolution. Proc Natl Acad Sci USA 84: 7846–7850

Takahashi T, Endo S, Nagayama K (1993) Stabilization of protein crystals by electrostatic interactions as revealed by a numerical approach. J Mol Biol 234: 421–432

Villoutreix B, Spassov V, Atanasov B, Herye G, Ladjimi M (1994) Structural modeling and electrostatic properties of aspartate transcarbamylase from Saccharomyces cerevisiae Proteins: Structure Function Genetics 19: 230–243

Weiner S, Kollman P, Case D, Singh U, Chio C, Alagona G, Proteta S Jr, Weiner P (1984) A new force field for molecular mechanical simulation of nucleic acids and proteins. J Am Chem Soc 106: 765–784

Yang A, Gunner M, Sampogna R, Sharp K, Honig B (1993) On the calculation of pK_as in proteins. Proteins: Structure Function, Genetics 15: 252–265

Yang A, Honig B (1993) On the pH dependence of protein stability. J Mol Biol 321: 459–474