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Folding interpenetration in a gliadin model: the role of the characteristic octapeptide motif

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Abstract

A model of a rheologically relevant protein, ω-gliadin, is proposed and studied in this work by means of molecular dynamics techniques. The model is based on an octapeptide repeat motif that is experimentally described as characteristic of that protein and as constituting it almost entirely. The initial molecular structure consisted of 20 such repeats. It was optimized and the dynamics developed along 980 ps, at dielectric constant $\varepsilon = 80$. Remarkable structural features were observed for the model built, such as an elongated, twisted tubular overall structure with a peculiar interpenetrating folding pattern, of a very regular character, organized strand formation, topologically segregated sites on the outer surface with an alternate hydrophilic/hydrophobic character and a hydrophilic inner cavity. Dynamics produced significantly more relaxed structures, but was not able to change the main geometric features presented by the original structure. Preliminary attempts of correlating some structural/dynamic aspects observed for the model with features of gliadin rheological behavior are presented.

Keywords: Interpenetrating protein folding; Bio-rheology; Computer simulation; Viscoelastic protein; Protein self-assembly

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1. Introduction

Wheat gluten is a very complex protein assembly with outstanding rheological features [1]. Such properties have been the basis of wide technological applications in food processes, specially aimed at the development of appropriate textural product characteristics [1–4]. The unique viscoelastic properties of wheat gluten have been empirically acknowledged since ancient times. More recently, innovative uses of gluten and of its constituents have been referred in the literature, such as those involving nanoparticle formation [5] for controlled drug delivery and development of thin edible films in food packaging [6].

From an academic point of view, such systems constitute very interesting models for the study of macromolecular self-association events and their relationships to solvation, flow behavior and colloidal properties. Despite gluten insolubility in water, the way through which water diffuses into that complex protein system during technological processes and the selective, partial solvation that may occur at particular sites in these instances [7–9] are decisive for the evolution of gluten's rheological properties.

Structural peculiarities of such protein structures seem to be also critically relevant in the context of a serious pathology of the gastrointestinal system, the celiac disease [10–12]. Of particular incidence among infants, the celiac disease involves an immunological intolerance to gluten, leading to a devastating nutritional condition of the patients. The antigenic role played by those complex protein structures is thought to be related to some unusual structural characteristics.

Several protein fractions can be isolated from wheat gluten [13]. Their occurrence as part of a very complex supramolecular structure, however, has usually hindered a more detailed structural characterization so far. Those more amenable to such an approach could perhaps be found in the gliadin group, a heterogenous protein fraction, whose components are grouped together by their common solubility in alcohol/water mixtures. They are characterized by a relatively lower molecular weight range (30–60 kD) as compared to that of the glutenins ($\sim$ millions kD), the alcohol-insoluble component of gluten. They also differ from the glutenins by the absence of intramolecular disulfide linkages. Four main sub-fractions (α, β, γ and ω), varying in size and composition, are chromatographically identifiable in gliadin [13].

Very intriguing characteristics have been described for gliadin of the ω group [8]. Its amino acid composition is mainly represented by only three amino acid residues, namely, glutamine (Q), proline (P) and phenylalanine (F), accounting for 40–50, 20–30 and 7–9%, respectively, of total amino acid composition. These amino acid residues are present in a peculiar octapeptide motif (PQQPFPQQ), which is repetitive along the molecule and responds for almost its entire composition. A few other amino acid residues appear in minor amounts. The repeat motif is referred as having antigenic properties [10,11].

In fact, glutamine and proline are consistently present as the major amino acid residues in most gluten's protein fractions [14], therefore suggesting their relevant role in the protein structure and mechanical function. Other important structural features described for those systems include the prevalence of β-sheets and the occurrence of spiral structures based on β-turn motifs, referred for elastomeric proteins, among which barley hordein [14], and a group of wheat gluten proteins [15].

With the purpose of creating a representative model for one of such proteins, we focused our attention on the intriguing occurrence of the octapeptide sequence in ω-gliadin and built a protein structure containing several of such repetitive moieties. We chose the model to be monomeric at this stage, since we were interested to follow the peculiarities possibly related to the occurrence of the octapeptide motif. In the molecule that we built, molecular extension was made sufficiently large to contain as many as 20 of such octapeptides.

2. Methodology

A 160-residue protein structure was built. It can be represented as $[octa]_{20}$ where octa stands

for PQQPFPQQ. The model was built with the Sybyl (Tripos, Inc.) software [16]. So as not to impose any particular conformational restraints to the initial molecule built, the amino acid residues were used in their default configuration. Such a configuration corresponds to ω (O=C–N–H) dihedral angle equal to 180° (trans configuration of the peptide bond) for all residues; ϕ (C–N–C_α–C) dihedral equal to −75° for the prolines and 180° for the glutamines and phenylalanines; and ψ (N–C_α–C–N) dihedral equal to 0° for the prolines and 180° for the glutamines and phenylalanines. Uncharged molecular endings were applied in the model. An academic program, (THOR) [17], based on GROMOS multiple body force field [18], was used for geometry optimization and for the molecular dynamics. Structure relaxation was achieved with optimization procedures that made use of the steepest descent followed by the conjugate gradient algorithms [19]. Molecular dynamics was initiated with a gradual heating stage, reaching 298 K after 10 ps. Dynamics was then followed at 298 K for an overall period of 980 ps. For the first 200 ps, the temperature was maintained by a direct re-scaling of velocities. For subsequent times the system was weakly coupled to an external heat bath, with relaxation time of 0.1 ps [20]. Kinetics and potential energies were recorded at every 0.2 ps and atomic coordinates and velocities at every 1.0 ps. The leapfrog algorithm [21] was used to perform the integration of the atomic trajectories with a time step of 0.5 fs. The united atom model was used for hydrogen atoms bound to aliphatic and aromatic carbon atoms, with explicit representation for all other hydrogen atoms. Such an approach has been successfully adopted in previous modeling work developed with THOR, including a 25-residue long signal sequence peptide [22] and β-casein, a 209-residue long protein [23].

Sixty-four ϕ (C–N–C_α–C) and ψ (N–C_α–C–N) dihedral angles were independently monitored at every 0.2 ps along the dynamics. Residues monitored extending from P65 to Q96, comprised four octapeptide sequences altogether. That corresponds to a molecular region that goes from octapeptide sequence number 9 to number 12, representing an extensive internal region of the molecule, with three sequence interconnections included. These sequences are denoted sequence A (from P65 to Q72), B (from P73 to Q80), C (from P81 to Q88) and D (from P89 to Q96). The reason for focusing on such an internal region is that it is less amenable to fluctuations than one would expect should occur at the molecule ends, due to lesser degrees of freedom in the former case. We expect the region chosen to be representative of the main events related to the octapeptide sequences and their interconnection sites in the molecule.

Graphical display of molecular structures was done with the RasMol program [24].

3. Results and discussion

3.1. Analysis of the molecular dynamics

3.1.1. The original structure generated: an interpenetrating folding pattern of regular character

Fig. 1 depicts the initial structure obtained for the gliadin model that was built. Only the polypeptide backbone is shown in that figure. Among its noticeable features, the most impressive is the interpenetrating folding pattern of remarkable regularity, as well as the peculiar occurrence of two rows of parallel strands that are anti-parallel in respect to each other. The interpenetration follows a consistent route, occurring by means of knotted loops at every three polypeptide stretches. The interior of the structure appears as a cavity, of an approximate twisted tubular geometry. In fact, the overall structure presents a twisted character. Fig. 2 shows a space-filling model of the same structure, displayed at different positions so as to allow a better visualization of its outer surface characteristics. It can be observed that regular protruding formations are apparent on the molecular surface and that they occur in a regular twisted mode, relative to each other. One can identify four main rows of such formations. In Fig. 3, one can observe that two of such rows,

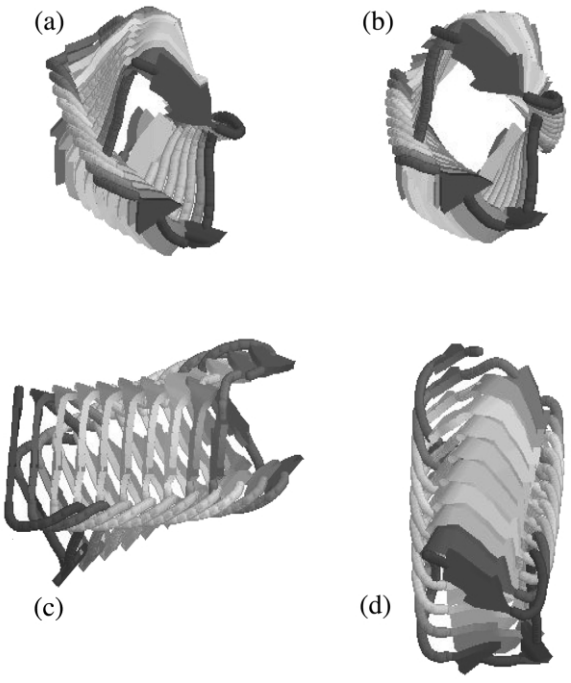

Fig. 1. Original structure (backbone only) for the $[octa]_{20}$ gliadin model at different views, showing its interpenetrating folding pattern. Two rows of turns (knots) and two rows of parallel strands, antiparallel to each other, can be visualized, as well as inner tubular cavity. A twisted overall structure can be clearly observed.

lying on opposite surface regions of the molecule, correspond to the alignment of phenylalanine residue side chains, located at the knotted interpenetrating loop region of the polypeptide backbone. The other two, also extending themselves on opposing surface regions, correspond to glutamine side chains and are located at the strand region. That defines an alternate polar (Gln)/non-polar (Phe) pattern of four strings on the outer molecule tubular surface. The inner cavity of the macromolecule, on the other hand, comprises glutamine residue side chains only, forming a totally hydrophilic environment in the interior of the molecule. A very regular set of hydrogen bonding was observed there (not shown). Model dimensions are as follows: maximum diameter (taking side chains into account) = 25 Å; internal tubular diameter = 15 Å; and length = 34 Å. Fig. 4 displays the front view of backbone structure for the first two octapeptide repeats, with residue identification. That should allow one to find the correspondence between residue position in the octapeptide sequence and its secondary structure, as displayed in Fig. 1b, Fig. 2a and Fig. 3a.

It can be verified that the above described regular interpenetrating folding pattern is already formed after a few octapeptide sequences and can be extended indefinitely, maintaining the very same folding pattern as the chain is elongated (Fig. 5). Minimum requirement for the establishment of interpenetration is three octapeptide sequences, as shown in Fig. 5, although the characteristic folding pattern will be typically recogniz-

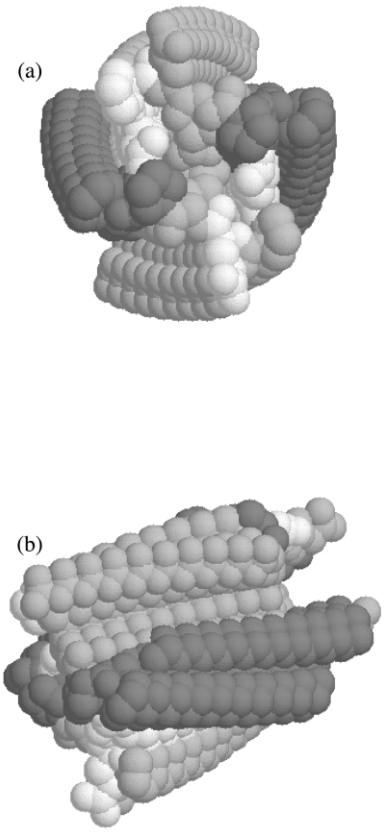

Fig. 2. Space filling original structure for $[octa]_{20}$ gliadin model: (a) front view; (b) side view.

able after a few more of them are present. Such a folding pattern, with interpenetration of the polypeptide backbone and the formation of regular entangled knots has not ever been described for globular, water-soluble proteins or for other structuring proteins described to date, to the best of our knowledge. The structure originally generated for that gliadin model comprises indeed a remarkable molecular architecture with aspects

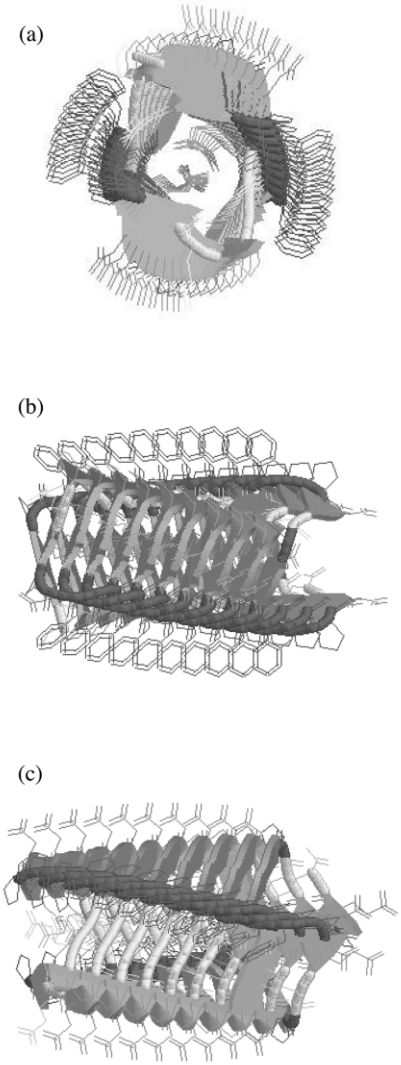

Fig. 3. Original structure for $[octa]_{20}$ gliadin model, including amino acid residue side chains.

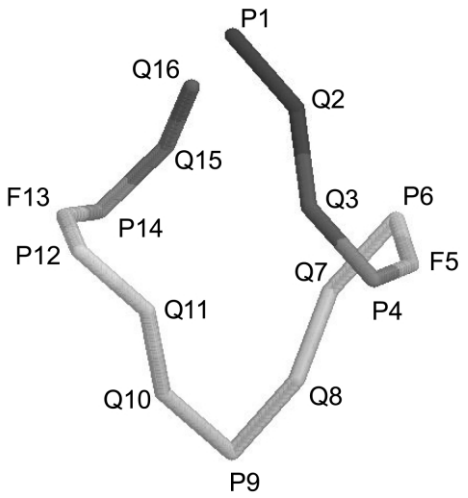

Fig. 4. Backbone structure display of two sequential octapeptides, from P1 to Q16, with residue identification. The structure is displayed in front view, in an approximately similar position to those represented in Fig. 1b, Fig. 2a, and Fig. 3a, so as to allow a comparison between residue position in the octapeptide sequence and model overall structure.

such as regularity and interpenetrating folding character, which may represent an important differential aspect for that protein category.

3.1.2. The evolution of the dynamics: maintenance of the general folding pattern

It was observed that folding interpenetrating character is maintained unchanged throughout the dynamics. However, the perfect alignment of residue side chains and the consequent regular strand formation of the original structure give place to more relaxed structures, as expected. (Figs. 6 and 7). In fact, main conformational changes occur at the optimization stage and are maintained afterwards, along the thermalization and the dynamics. Elements of symmetry, however, are persistent and can be recognized throughout the modeling. The same can be said about main features at the outer surface of the molecule and the overall twisted character of the structure (Fig. 7).

Structures developed at dynamics times where main variations in energy and dihedral angles were observed (70 and 170 ps — see Figs. 8 and

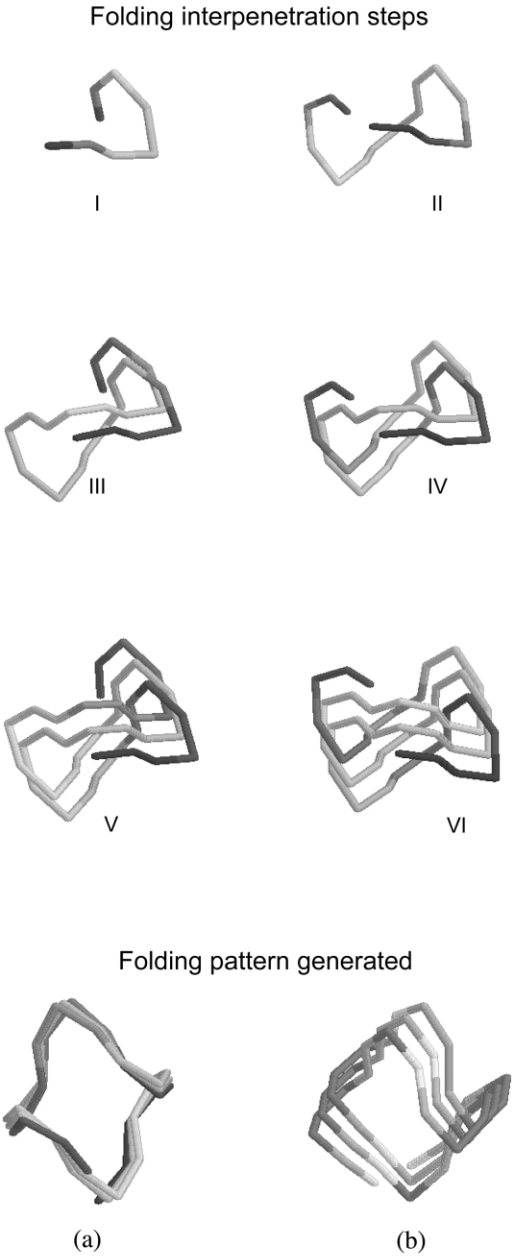

Fig. 5. Interpenetration of backbone folding with chain elongation, as number of octapeptide sequences is increased from one (I) to six (VI). A and B depict final views for the $[octa]_6$ protein built, at two slightly different perspectives.

9) are included in Fig. 6. It can be seen that the main overall molecular structure is maintained even in these instances. Energy changes, therefore, seem to be related to local structural chain accommodation that does not appear to interfere with molecular folding pattern or main geometrical features of the molecule as a whole.

Main features observed for a side view of the gliadin model along optimization and dynamics comprise, as depicted in the various pictures in Figs. 6 and 7, a distorted shape with two lobes of enlarged molecular volume, represented by the two molecular endings, and a central, apparently stretched region. Lobular domains could be described as prolate ellipsoids, if taken isolated.

Final structure generated after 980 ps dynamics is depicted in Fig. 7 in greater detail, including amino acid residue side chains. Maintenance of molecular features described above can be observed here. Molecular front and side views still keep main original features. A top view is included and it can be seen that the 'stretched' central region of the side view has in fact a similar ellipsoidal shape as the two ending lobes, distorted 90° relative to them. That corresponds to a molecular structure that has undergone a twist. The elongated, alternate hydrophobic/hydrophilic domains follow a spiral path at the molecular surface (Fig. 7f). Phenylalanines are found there preferentially located at the turns (knots), just as in the original structure. They appear to keep segregated from the glutamines, which are found mainly located at the residual strands or at non-structured regions of the molecule.

From results of this work taken together with results from a parallel work being developed by the authors, it seems that the peculiar interpenetrating folding, apparently characteristic of proteins containing the octapeptide sequence and consistently maintained throughout the dynamics, is intrinsically related to the proposed sequence and basically insensitive to environmental conditions.

3.1.3. Analysis of molecular parameters (ϕ and ψ dihedral angles) and energies along the dynamics

Rearrangements of the polypeptide backbone can be revealed by the observation of ϕ and ψ dihedral angle variations (some of which are dis-

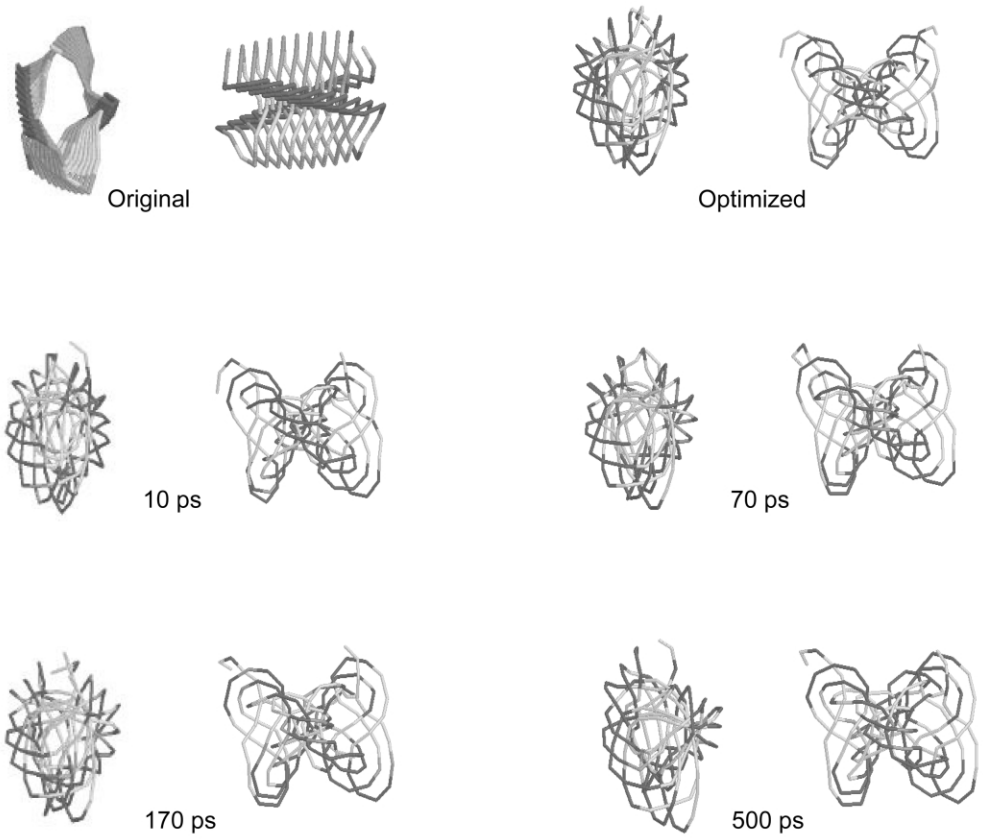

Fig. 6. Evolution of molecular geometry for $[octa]_{20}$ gliadin model: from the original structure, through optimization, thermalization stage (10 ps), and along different times of the dynamics.

played in Fig. 8) and of the structures produced along the modeling work (Figs. 6 and 7). For the gliadin model studied here, such rearrangements were mainly limited to the first 200 ps of the dynamics, affecting only part of the residues. Very stable angle values were observed after that time stage for nearly all cases (except for a change at 870 ps involving two out of 64 dihedral angles monitored) and well before that for many residues (Fig. 8). Actually, nearly half of the monitored residues did not present any significant dihedral angle change throughout the dynamics.

The evolution of the potential and total energies followed a pattern with two distinct regions of abrupt decrease at approximately 70 and 170 ps followed by a smooth decrease until approximately 200 ps (Fig. 9). From that point onwards, total energy is maintained at rather stable values up to 870 ps, where a slight decrease is again observed. The decrease in potential and total energies occurs at dynamics times where the most significant changes in dihedral angles were observed for some sensitive residues. That can be observed for instance in Fig. 8, where some dihedral angles monitored are displayed.

3.2. On the possible correlations between structural features of the model and mechanical behavior

The peculiar interpenetrating folding pattern observed, like that of a knot, is compatible with and suggestive of the idea of chain movements in a stretching-squeezing mode, as if a knot were being made tighter or looser. That could occur

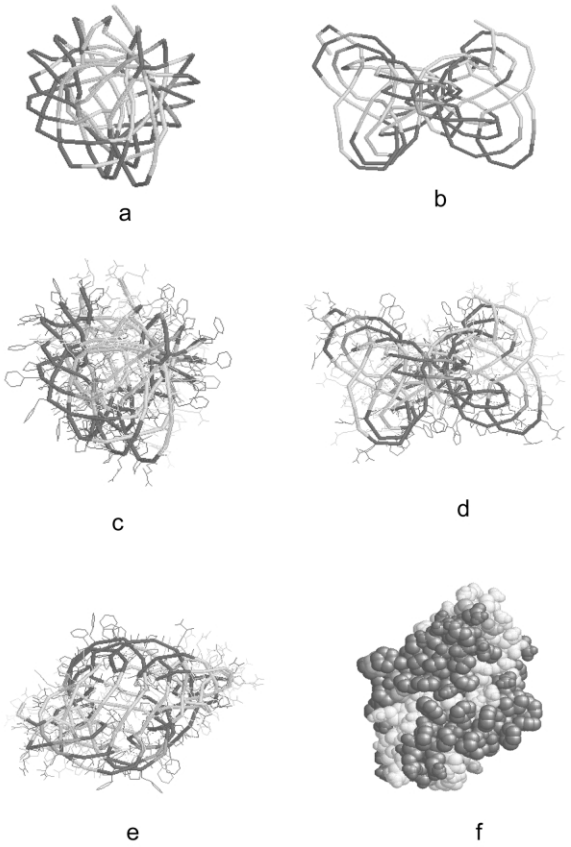

Fig. 7. Molecular structure for $[octa]_{20}$ gliadin model after 980 ps of dynamics: (a) front view; (b) side view; (c) front view with residue side chains included; (d) side view with residue side chains included; (e) top view; (f) space filling model of final structure.

without loss of the protein basic folding pattern due to its interpenetrating loop character, which would represent a relatively flexible structural constraint.

There is no correspondence to that in the globular protein family, where folding patterns are not interpenetrating, leading necessarily to much narrower limits of existence of a characteristic three-dimensional structure. Exceptions to that would be proteins bearing disulfide bonds; however, in these cases, constraints are covalent linkages and no similar slippering displacements of the kind described above would be feasible for the molecule as a whole. Since in gliadin the primary sequence is basically represented by the recurrent appearance of the same three amino acids in a characteristic pattern, it is reasonable to consider the possibility of occurrence of transient intrachain contacts, likely to involve pairs of similar residues at nearby sites, statistically accessible in the chain structure. For the gliadin model investigated, it is apparent that its folding could be maintained even when considerable atom displacements from their local minimum energy positions have occurred.

Complete disentanglement of such a structure would be a statistically unlikely event, since it would require a coordinated chain movement in a particular route and direction within a microenvironment considerably constrained by interpenetrating knots and stabilized by a complex set of hydrogen bonding (not shown here). Although such a putative reptation movement could in principle be envisaged as analogous to that occurring in synthetic polymer systems [25,26], it seems to us that the peculiarities of structure for the model studied here would most probably restrict chain movements to reptation–retraction steps of limited amplitude. Also, other moieties associated to gliadin in the gluten complex may represent further restraints to complete chain disentanglement.

One could reasonably argue that if unfolding is an unlikely event for the reasons mentioned above, the folding process should be as well unlikely, since the same geometrical constraints would apply, in principle. That would be true if folding were considered to occur from a stretched or random-coiled protein structure. However, if taken in the context of the translation process at the ribosome, the picture can be rather distinct. The step-by-step translation process could assist the nascent chain to adopt the appropriate chain angles as it is being generated. The interpenetrating pattern may be favored as the nascent chain stems from the ribosome tunnel (whose dimensions are 25×125–150 Å), along the translation process itself. Space constrictions represented by such a cavity could perhaps add further assistance along the process (as indicated, gliadin model cross section is approximately 25 Å). A chain slithering movement, continuously fed by the in-

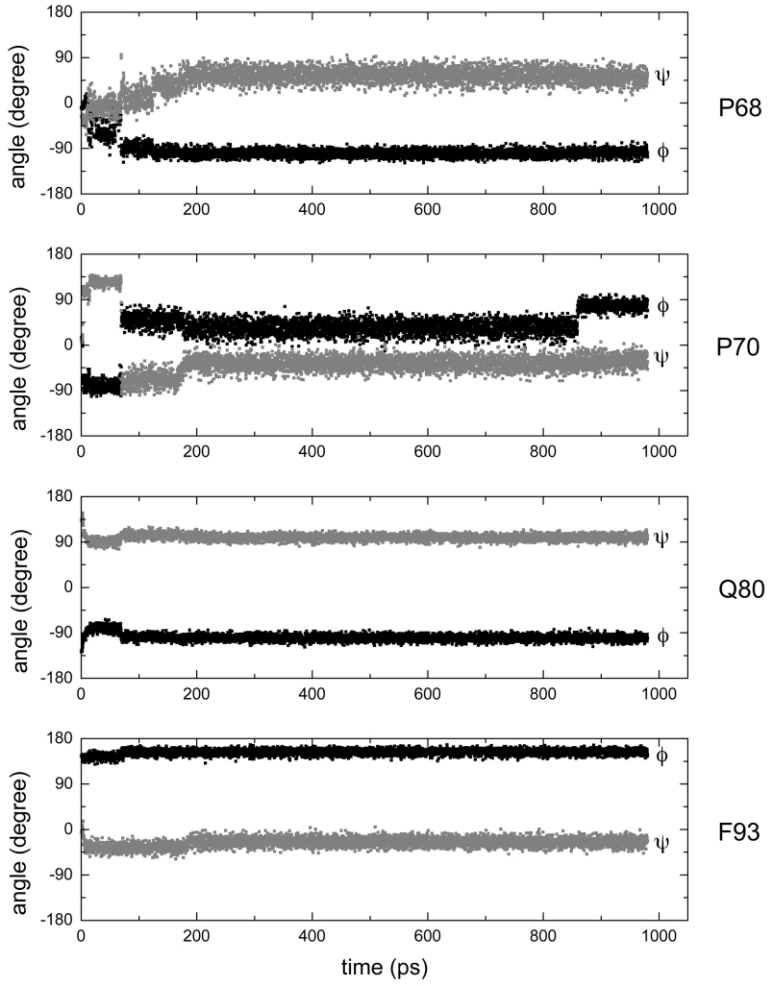

Fig. 8. Evolution of a few pairs of ϕ and ψ dihedral angles along the dynamics.

coming nascent polypeptide, would permit chain interpenetration to occur from the N-terminal end towards the C-terminal end, without involving the ribosome in the knot entanglement. Alternatively, small protein interpenetrated fragments could be released from the ribosome before further geometrical restrictions build up, and then be joined together by enzymatic action. In this respect, more experimental support from the molecular biology of such systems would be essential to allow further speculations on such aspects.

The peculiar string formation of alternate hydrophobic/hydrophilic character on the external molecular surface suggests the potential feasibility of the system for interaction with polar and non-polar ligands at topologically segregated sites. The inner cavity, however, is exclusively of hydrophilic nature, thus representing a possible preferential route for fast water diffusion in these systems. It could also represent an adequate bicontinuous medium in larger structures for the entrapment of gas during the formation of dough and in baking.

The force field applied in this work takes into account interatomic interactions only [17,22,23],

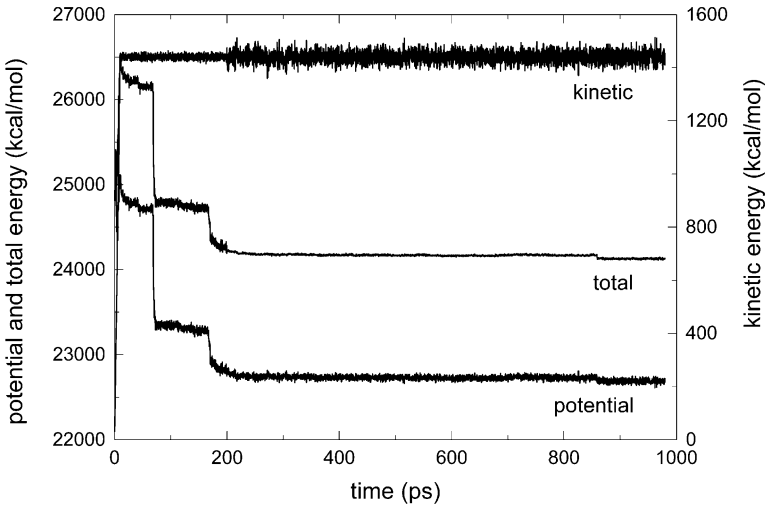

Fig. 9. Evolution of potential, kinetic and total energies along the dynamics.

not including any term on fields external to the molecule. It would be interesting to model such a structure under a shear force field, in order to observe the evolution of its dynamics in a situation where the molecular system is known to display viscoelastic properties in flow behavior. The intriguing twisted structure observed is very suggestive of a system that would be able to respond to shear forces in a multi-directional way, without easy rupture of its internal structure.

4. Concluding remarks

This work proposes an entirely new possibility for the space organization of a mechanically relevant protein, ω-gliadin, a prolamin from gluten. The modeling work spontaneously revealed a peculiar interpenetrating folding pattern, of very regular character, not known to occur for globular proteins and which is, in principle, compatible with the described rheological behavior for the gluten protein.

Detailed spectral analysis of gluten and its components has been limited by the inherent system complexity where states of aggregation and specific-intermolecular interactions are critical for structural identity. As a consequence of that, no high-resolution molecular structure has yet been known to any such protein systems, despite extensive and relevant structural work available to date on these systems, examples of which can be found in the literature cited [4,7–9,14,15,27,28]. Modeling work may be of relevance in this context [29,30], indicating structural tendencies for a given molecular system. We think that new insights into gluten proteins' structure may arise if folding interpenetration is included as a structural constraint, in a possible reassessment of existing spectral data and in the analysis of new incoming experimental data.

The model proposed in this work agrees with the consistent indication in the literature of the prevailing occurrence of turns and sheets in wheat prolamins [7,8,14,15,27] and with their ability to interact with polar and non-polar species [4]. Antigenic properties of such proteins, ascribed to the octapeptide repeat [10,11], could as well be related to this very peculiar, unorthodox interpenetrating folding, not displayed by any other protein system described to date, to the best of our knowledge. The original torsioned interpenetrating folding presented here is in fact a self-assembled molecular arrangement of a very peculiar spiral character. It is distinct, though, from other novel secondary structures, such as certain spiral

structures described for some elastomeric proteins where no folding interpenetration is present [14,15,29–31].

Differences concerning hydrogen bonding and specific solvation aspects would be expected to occur if the modeling procedure included explicit water molecules rather than a dielectric continuum, as was the case here. However, possible differences in this respect would not be expected to alter main points revealed by the modeling, such as the persistent interpenetrating folding.

On the basis of the current data available, we envisage that cereal proteins could contain whole sub-components or domains of different extents where such unconventional foldings are found.

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