

The probable structure of the protamine–DNA complex

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Abstract

A detailed molecular structure is proposed for the human protamine–DNA complex, which has hitherto been largely a mystery. The structure was created with virtual modeling software (AmiraMol), employing logical deduction as the primary investigative tool.

A β -sheet structure for the protein component is essentially mandated, as the alternatives can be decisively excluded.

A dimeric structure too is essentially mandated, since the cysteine residues of protamines P1 and P2 are invariably aligned in all species having both chains.

The cross-sectional and axial spacings of arginine guanidinium groups in this protein structure can be perfectly aligned with those of phosphate groups in DNA according to the DNA structure proposed by Wu. This is a non-helical structure, whose possible occurrence in certain plasmids has been suggested by experimental observations.

The unit cell of this protamine–DNA complex is essentially devoid of steric hindrances, and heavily favored by a multitude of ionic and hydrogen bonds.

The packing of adjacent “unit cells” of the protamine–DNA structure is based on a complex array of salt bridges, the mere existence of which is so fortuitous that it is virtually inconceivable that it comes about through a mere modeling “coincidence”.

The possible significance of the structure beyond the sperm cell is discussed.

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1. Introduction

While there exist some simple models of the complex between protamine and DNA in the sperm cell (Vilfan et al., 2004; Martins et al., 2004), there has never been, up to the present time, any detailed molecular model of this important structure. Although it is not doubted that the interaction between protamine and DNA is electrostatic, no published structure gives any clue as to precisely how the positively charged basic residues in protamine are to align with the negatively charged phosphate groups in DNA.

Protamine is not the most complicated, but, on the contrary, one of the most *simple* of the nucleoproteins. In view of the fact that a detailed molecular model exists for the nucleosome (Luger et al., 1997a, b), whose histone component has eight protein subunits and nearly an order of magnitude more atoms than protamine, the absence of

such a model for the far simpler sperm nucleoprotein is somewhat startling.

The protamine–DNA complex is apparently not going to be crystallized anytime soon, and thus a laboratory solution is not forthcoming. I have therefore turned to logic as a tool to attempt to deduce the structure. This has proven to be a most fruitful approach, as a thoughtful consideration of the characteristics of the protein and nucleic acid components leads strongly to some important structural conclusions.

2. Basic protein structure

Human protamine is a small protein, consisting of 2 chains (called P1 and P2) of about 50 amino acid residues each. Nearly half of these residues are arginine. It has never been doubted that the positively charged guanidinium groups of arginine bind electrostatically to the negatively charged phosphate groups of DNA, but a detailed

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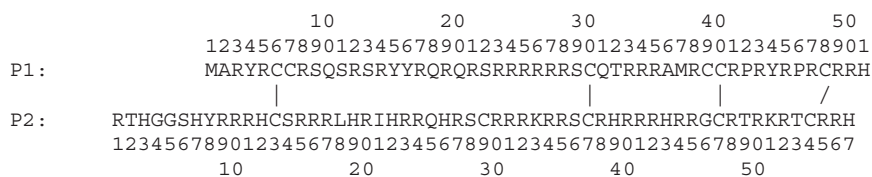


Fig. 1. Amino acid sequences of protamines P1 and P2. The vertical lines indicate the likely sites of disulfide bond formation, except for the slanted line at the C-terminal end, where a disulfide bond cannot currently be formed because of excessive distance between the cysteine residues.

structure which satisfactorily aligns these salt bridges has never been suggested.

The non-arginine amino acid residues are virtually all either basic (lysine, histidine) or chosen from among the group of amino acids capable of donating a proton for a hydrogen bond with a DNA phosphate group: tyrosine, cysteine, serine, glutamine, and threonine. Also found are rare instances of amino acid residues which deviate from these parameters, including leucine, isoleucine and proline. Concerning the order in which any of these residues occur in the amino acid sequence, no logical pattern can be clearly discerned, either in human protamine alone (Fig. 1), or by way of comparison between protamines of the many different species which have been studied. It would appear that nature is demonstrating here a sort of tolerance which might be a good role model of tolerance for human beings generally: where additional arginine residues are not needed (which, if present, would presumably result in a biologically inactive, quasi-crystalline structure), any of the above-referenced hydrophilic side-chain will suffice, in any order, and even an occasional hydrophobic group will be tolerated.

What is the structure of the protein component of sperm nucleoprotein? Protamine does not have enough hydrophobic residues to provide a basis for a core of a globular protein structure, which is therefore excluded. Nor are there any significant numbers of negatively charged acidic residues to neutralize a hypothetical globular core stabilized by salt bridges.

The α -helix also seems an unlikely solution, and if we were to assume the Watson-Crick structure, an α -helix becomes impossible, since the many positive charges in protamine cannot thereby be made to follow the negative charges in DNA through helical space. I say this after having spent a goodly amount of time in futile attempts to anastomose DNA to virtual models of the α -helix constructed with the protamine amino acid sequence. Although my efforts can perhaps be dismissed as inconclusive, the best evidence against the α -helix as a structure for protamine is that in 50 years, no one else has succeeded in making it work either.

As we shall see, however, a β -sheet provides a very workable solution, provided that one is prepared to forego the helical twist in DNA.

3. Probable dimeric structure

All protamines have several cysteine residues in each chain. In human protamine (Fig. 1), as well as in the

protamines of other species, there is a striking tendency for these cysteine residues to lie adjacent to one another when P1 and P2 are laid out side-by-side. Knowing the important role generally of cysteine in the formation of disulfide bonds, the only sensible first assumption is that these residues are there for that purpose.

Once these aligned cysteine residues are assigned to be the sites of disulfide bonds, we can begin to do some calculations. The estimated length of two fully extended arginine residues, one from P1 and one from P2, when separated by the length of a disulfide bond, is about 14 Å. The phosphate-to-phosphate cross-duplex dimension in the Watson-Crick double-helix (and all other duplex DNA structures I am aware of) is about 20 Å. If one allows 3 Å for salt bridges between the positively charged guanidinium groups of arginine and the negatively charged phosphate groups of DNA, the fit appears to be very good (Fig. 2A).

In the β -sheet, alternating amino acid residues will be on the same side of the sheet. Therefore, in a length of protamine adjacent to DNA, the arginine-to-arginine distance on the side close to the DNA would be the distance between *every other* amino acid residue, namely about 6–7 Å (Fig. 2B–C), which just happens to be the same spacing as the intrastrand longitudinal distance between adjacent phosphate groups in DNA, when measured along the sugar-phosphate backbone.

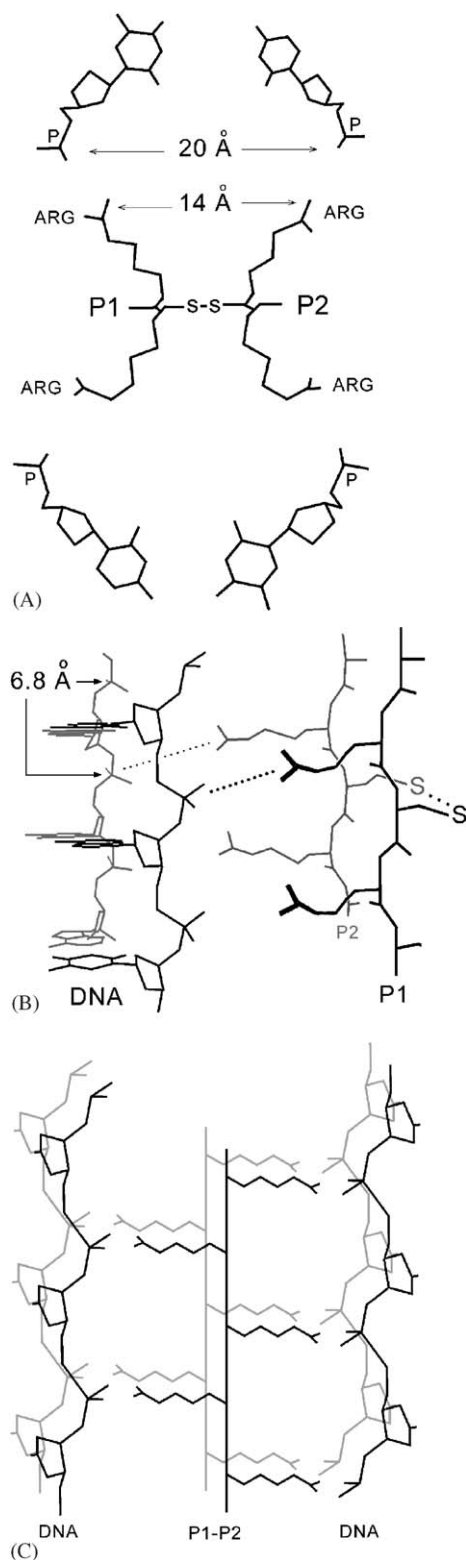
Rather than to treat these fortuitously favorable spacings as mere coincidence, I have elected to regard them as indications of the means whereby the positive charges on protamine may be aligned to the negative charges on DNA in sperm nucleoprotein.

4. DNA structure

Since a β -sheet has no helical twist, we must choose from among several hypothetical non-helical DNA models to accommodate to it. The side-by-side models of Rodley (Rodley et al., 1976; Milane and Rodley, 1981) and Sasisekharan (Sasisekharan et al., 1976, 1978), and the paranaemic structure of Delmonte (Delmonte and Mann, 2003), cannot be ruled out, but anastomosing them to a β -sheet would be difficult. On the other hand, the “straight ladder” structure of Tai Te (Wu, 1969; Wu and Wu, 1996) works very well with protamine. The Wu structure is only implied in his published works; no physical or virtual model exists.

Wu has always believed that at 92% humidity, the X-ray diffraction pattern of DNA reveals not a duplex with

3.4 Å base-pair spacing, but a tetraplex consisting of two mutually intercalated duplexes each having 6.8 Å base-pair spacing (Wu, 1969). In the cell, i.e. at 100% humidity, Wu believes that this structure untwists into what he refers to as a “straight ladder” (personal communication). This structure, it turns out, can be made to accommodate itself to a protamine β -sheet almost effortlessly.



Although Wu's view of DNA structure has been generally held to lie in the realm of interesting theory, he has nevertheless gone on to present striking data suggesting the possibility that the structure may actually exist in at least two plasmids (Wu and Wu, 1996).

5. Materials and methods

The virtual model of protamine–DNA structure to be described was created entirely with AmiraMol, kindly made available by Mercury Computer Systems, Inc.

5.1. Details of the model

5.1.1. Longitudinal considerations

Fig. 2A–C shows the essential features of our model, as well as can be depicted in two dimensions (for 3D structures, see Biegeleisen, 2005). It consists, first of all, of two polypeptide chains, protamines P1 and P2, placed vertically with respect to the z -axis, and connected by disulfide bonds (at the sites indicated in Fig. 1). Associated with the protein component are two Wu “straight ladder” DNA duplexes, each one binding to one of the two approximately equivalent faces of the protamine dimer by ionic bonds between arginine side-chains and DNA phosphate groups (for graphic clarity Fig. 2B, which is a nearly literal projection, shows only one of the DNA duplexes; 2C shows both in simplified schematic form).

The structure depicted schematically in Fig. 2C may be regarded as the “unit cell” of sperm nucleoprotein, consisting of two protamine chains ionically bound to an equivalent length of DNA on two sides; i.e. 108 amino acids and 58–59 base pairs of DNA.

Fig. 2. Three views of protamine–DNA structure. (A) Top view, showing P1 and P2 connected by a disulfide bond, and a pair of Wu “straight ladder” DNA duplexes, one at the top and one at the bottom of the picture. Note that the bases, which look like base pairs in this projection, are actually 3.4 Å apart axially, due to the anti-parallel nature of duplex DNA (see panel B). The distance between two extended arginine residues (“ARG”) is about 14 Å, and the cross-duplex distance between DNA phosphate groups (“P”) is about 20 Å. This spacing permits the formation of 3 Å salt bridges (not explicitly drawn in) between the arginine residues of protamine and the phosphate groups of DNA on either side of the protamine dimer. (B) Side view showing 6.8 Å spacing between base pairs. The every-other-residue spacing between amino acid residues is also 6.8 Å. A disulfide bond is included to show the “cis” type of relationship between P1 and P2. For purposes of graphic clarity, only two of the four arginine-phosphate salt bridges are drawn in (dotted lines), and only one DNA duplex is shown. (C) Highly schematic representation of the “unit cell” of the structure, which contains a P1–P2 dimer and a pair of DNA duplexes, one on each side of the protamine. Only arginine residues are shown. Although there is only one region of protamine which actually contains [almost] exclusively arginine residues (P1, residues 23–28 and P2, residues 40–45), the manner shown for ionic binding of arginine to phosphate is essentially the same wherever arginine occurs in the structure.

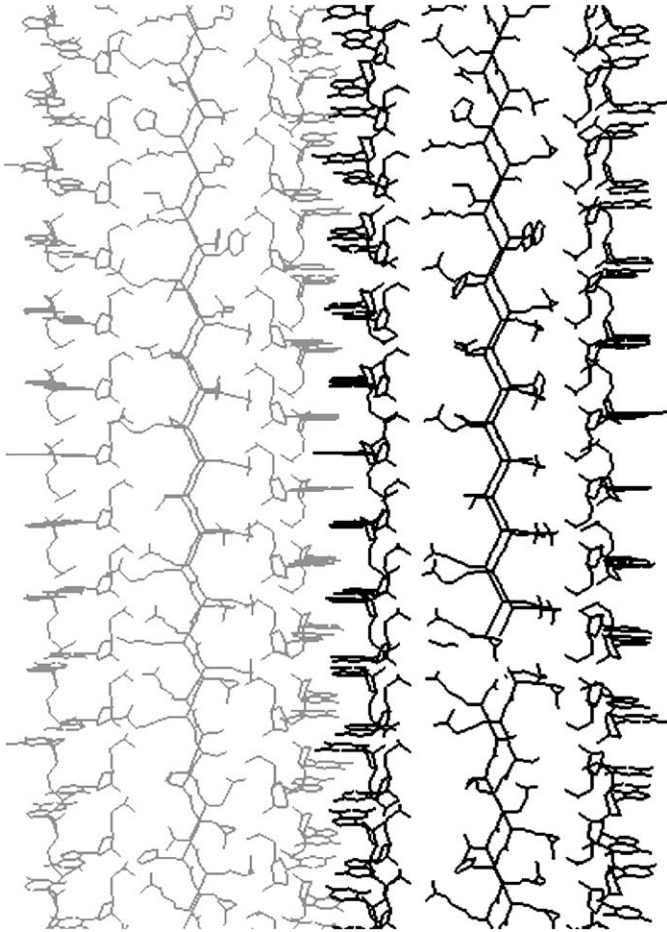


Fig. 3. Intercalation of adjacent DNA strands. Two “unit cells”, each consisting of P1–P2 plus two DNA duplexes, are shown. One “unit cell” is colored black, the other gray. They are “bound” by the hydrophobic bond resulting from mutual intercalation of base pairs, giving a final inter-base spacing of 3.4 Å. Note that the “unit cells” are pulled apart slightly for graphic clarity; if fully intercalated, the drawing would be almost unintelligible.

The DNA base-pairs of adjacent “unit cells” are mutually intercalated, giving a final base-pair spacing of 3.4 Å (Fig. 3).

5.1.2. Protein backbone

The bond lengths and bond angles in the polypeptide backbones of our protamine–DNA complex (Fig. 4, top) are essentially standard. The disulfide bond alignment more-or-less mandates a parallel structure for P1 and P2, analogous to that seen in α -keratin.

In principle, P1 and P2 can have either a “cis” type of relationship, i.e. one in which the peptide backbones have the same orientation, or a “trans” relationship, in which one of the polypeptide backbones is rotated 180° relative to the other. The presumed requirement for disulfide bonds, however, strongly favors the “cis” relationship (Fig. 4, bottom).

The inter-residue amino acid spacing was made to accommodate to the 3.4 Å inter-base spacing seen in the original Watson-Crick DNA structure. For the protein

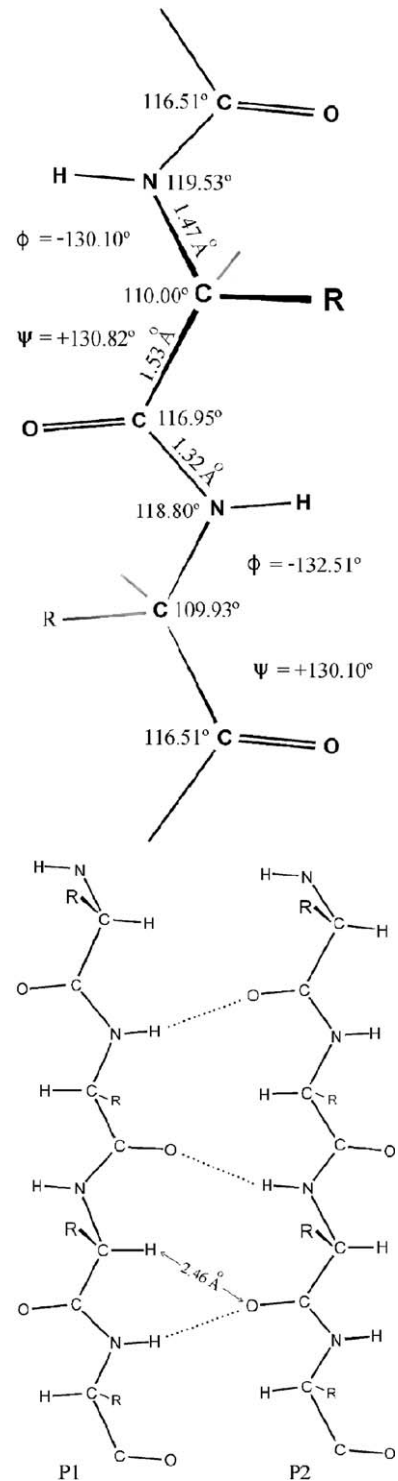


Fig. 4. Details of protamine peptide backbone. *Top*: Bond lengths, bond angles and dihedral angles. Note that there are small differences between adjacent residues (e.g. ϕ is either -130.10° or -132.51°). These differences are merely mathematical subterfuges which were necessary for the creation of a dipeptide unit to “clone” along the z -axis. *Bottom*: P1–P2, showing the “cis” type of relationship. The three hydrogen bonds (dotted lines) are each 3 Å. The 2.46 Å spacing shown, between HC α of P1 and O of P2, is the only steric hindrance in the backbone. It is essentially insignificant, violating the Van der Waals radii by only 5%.

β -sheet, this comes about when the ϕ and ψ angles are set to approximately -130.5° and $+130.5^\circ$, respectively (the actual values in adjacent residues had to differ slightly for virtual modeling purposes; see Fig. 4, top). These ϕ and ψ angles are well within the energetically most-favored region for β -sheets in the left upper quadrant of the Ramachandran Plot.

Recall that on either side of the β -sheet, the relevant spacing is between every-other-residue, i.e. 6.8 \AA (Fig. 2).

Our structure has three disulfide bonds, with standard S-S bond lengths of 2 \AA , and CB-S-S angles of 104° . These bonds introduce minor distortion to the local architecture of the polypeptide backbone described in Fig. 4, but these are readily corrected with small dihedral angle adjustments of adjacent amino acid residues, so that the ionic bond lengths between arginine and DNA phosphate remain close to 3 \AA .

Human protamine P1 has two proline residues. Since proline is not present in all mammalian protamines, and has no “standard” position when it is present, I assume that it is not there to generate a “kink”. Rather, I have treated proline as a programming nuisance, endeavoring to maintain the straightness of the polypeptide backbone as much as possible in its vicinity. The result is best seen in the virtual model (Biegeleisen, 2005).

5.1.3. DNA structure

The bond lengths and angles of our DNA sugar-phosphate backbone are shown in Table 1, and a flat projection in Fig. 5. For modeling purposes, the backbone is perfectly vertical, and the planes of the bases perfectly perpendicular with respect to the z-axis. There is no helical twist.

Table 1
Detailed specifications of DNA structure

Residue spacing (\AA)	6.80
Bond lengths (\AA)	
P–O5'	1.62
O5'–C5'	1.44
C5'–C4'	1.50
C4'–C3'	1.52
C3'–O3'	1.40
O3'–P	1.64
Bond angles	
P–O5'–C5'	117.73°
O5'–C5'–C4'	110.44°
C5'–C4'–C3'	108.79°
C4'–C3'–O3'	108.92°
C3'–O3'–P	119.60°
O3'–P–O5'	106.44°
Dihedral angles	
α (O3'–P–O5'–C5')	-174.03°
β (P–O5'–C5'–C4')	95.50°
γ (O5'–C5'–C4'–C3')	170.94°
δ (C5'–C4'–C3'–O3')	130.95°
ϵ (C4'–C3'–O3'–P)	-161.57°
ζ (C3'–O3'–P–O5')	-53.97°

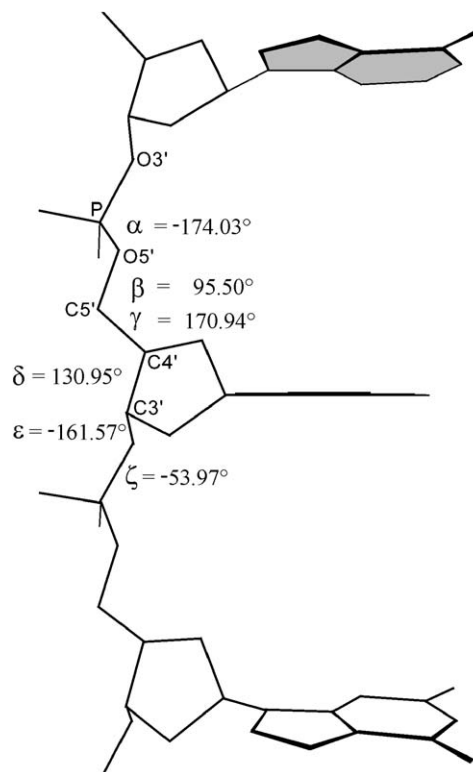


Fig. 5. Two-dimensional projection of DNA backbone, showing the large (i.e. 6.8 \AA) inter-residue spacing, and the perpendicular relationship between the backbone and the plane of the bases.

The inter-base distance is 6.8 \AA , which allows perfect alignment of DNA phosphate groups with arginine side-chains on either side of the protamine β -sheet (Fig. 2B–C). After intercalation of the base pairs of adjacent DNA duplexes (Fig. 3), the final base spacing is 3.4 \AA .

Establishing dihedral angles for the DNA sugar-phosphate backbone is an immensely complex problem, having, in principle, an infinite number of solutions. The backbone structure presented here was created by laborious trial-and-error, and is unlikely to be the best one possible. That notwithstanding, it has, in total, only one moderately objectionable steric hindrance. This involves ribose hydrogen atom $2H2'$ and the pyrimidine hydrogen atom $H6$ (which are not shown in the PDB structure file; a complete model with all hydrogen atoms is available from the author on request). These atoms are separated by a scant 1.6 \AA , which, nevertheless, passed the Protein Data Bank's steric hindrance filter. Therefore, and since furthermore this is the only significant steric hindrance in the entire protamine–DNA structure, I have left it uncorrected, as the structure cannot, in any event, be perfected in the absence of corroborating biological data.

Our O3'–P bond (1.64 \AA) is a bit long, and the 106.44° O3'–P–O5' angle a bit large compared to other published DNA structures (where O3'–P–O5' angles average around 102° , with a range of about 86° to 108°). Aside from these

relatively minor issues, the DNA backbone in protamine–DNA is chemically unremarkable. I should note that I have also created a Wu type “straight ladder” structure in which every DNA backbone bond length and angle is identical to that found in “classic” Watson-Crick DNA (not shown), but adjacent “unit cells”, created thusly, may not pack quite as well in the sperm head as the “unit cells” of our structure (see below).

Our ribose rings have been left as C2'-endo, for lack of any evidence to support any other conformation at this time.

The bases, both purines and pyrimidines, are in the high anti range with χ values of about -100° , which places them in a relatively energetically favorable part of the energy vs. χ curve.

5.1.4. Three-dimension arrangement of protamine–DNA chains

The “unit cell” of our protamine–DNA structure is a P1–P2 dimer ionically bound to two DNA duplexes (Fig. 2C). The three-dimensional arrangement of adjacent unit cells is shown in Fig. 6 (corresponding to PDB file 2AWS; Biegeleisen, 2005). The unit cell can bind to adjacent unit cells at either end by mutual intercalation of DNA base-pairs, giving rise to linear rows of alternating columns of DNA and protein.

How would adjacent rows interact with one another? At the outset of this project, I had no theory to deal with this aspect of protamine–DNA structure. Fortunately, none was needed. It turns out that adjacent rows, when displaced by half of a unit cell, fit together like puzzle pieces (Fig. 6), bound together by square arrays of fortuitously placed salt bridges.

It is impossible for me to imagine that this fit, which completes this structure with a degree of perfection which was not anticipated, is a mere modeling coincidence, but rather that it is an indication of the probable arrangement of DNA and protein in the sperm head.

6. Volume of the structure

The volume of a “unit cell” of the structure may readily be calculated from the PDB file 2AWS (Biegeleisen, 2005), depicted graphically in Fig. 6, which is a top view, looking down the z-axis. The drawing is virtually rectilinear with respect to the x-y coordinate axes, which, however, are not drawn in. The length of the unit cell is the y-axis distance between analogous atoms in comparable DNA or protein chains, e.g. L-to-F, or G-to-A (these two measurements giving similar but non-identical figures), and the width is the x-axis distance, e.g. L-to-V. The height is simply the length of protamine. The average figures are: length 26.5 Å, and width 18.0 Å. The height, i.e. the length of protamine in this model, is 205.7 Å. The volume, therefore, is

$$26.5 \text{ \AA} \times 18 \text{ \AA} \times 205.74 \text{ \AA} = 98,138 \text{ \AA}^3 = 98.14 \mu\text{m}^3,$$

which we may round off to $100 \mu\text{m}^3$.

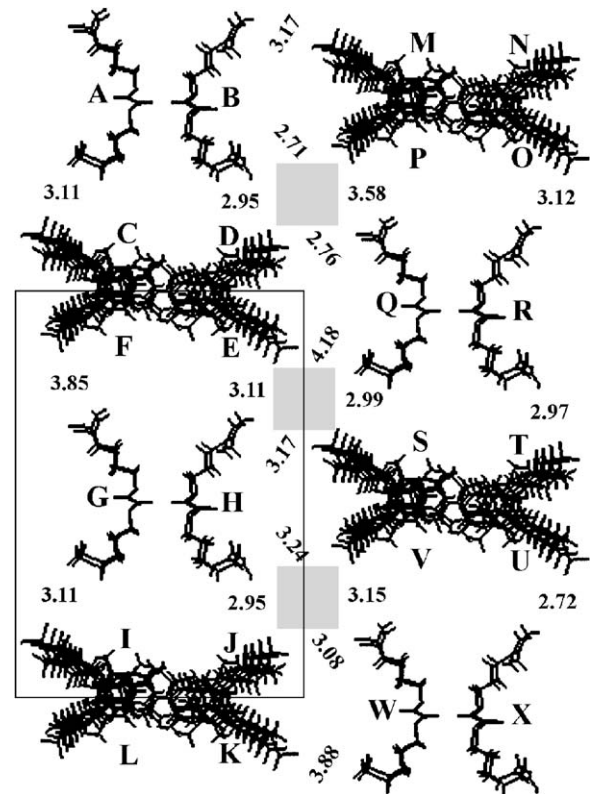


Fig. 6. Top view, showing packing of adjacent “unit cells”. The “unit cell” (one of which is enclosed by the large rectangle) is a fairly rectangular-shaped group of protein and nucleic acid chains (e.g. E–F–G–H–I–J), consisting of a P1–P2 dimer connected to a pair of “straight ladder” (i.e. non-helical) DNA duplexes (F–E and I–J) by salt bridges. Along the short sides of the rectangle (FE and IJ) the binding to the adjacent “unit cells” is hydrophobic, resulting from mutual intercalation of base pairs at the borders of the cells (the “X”-shaped objects in the diagram, e.g. CDEF and IJKL) (see also Fig. 3). This gives rise to linear rows of alternating columns of DNA/protein/DNA/protein/etc. (e.g. chains AB/CDEF/GH/IJKL), which could conceivably continue throughout the full length of the sperm head. Adjacent rows (e.g. chains M through X), displaced by a distance corresponding to half of a unit cell, fit together like puzzle pieces, according to a repeating square array pattern of salt bridges (marked by gray squares). The chain identifiers of the chains constituting the corners of the squares are BPQD, EQSH and HVWJ. Each of the four sides of these squares is a nearly perfect (i.e. approximately 3 Å) ionic bond between a positively charged arginine side chain and a negatively charged DNA phosphate group. The numbers between each pair of oppositely charged atoms represent the lengths of the salt bridges between them (as per the model currently posted on the Protein Data Bank, accession #2AWS). The drawing is an almost-literal projection of the actual virtual structure, adjusted only for perspective, to accommodate the 3-D structure to a flat manuscript page.

A sperm head contains the haploid amount of DNA, about 3×10^9 base pairs. Although it may not be perfectly clear from the two-dimensional drawings herein, the structure contains 59 base pairs of DNA for each protamine P1–P2 dimer. There are therefore $3 \times 10^9 / 59 \approx 50$ million “unit cells” in total, each with a volume of $100 \mu\text{m}^3$. The total predicted volume of sperm head DNA complexed to protamine, according to our model herein described, is therefore $5 \mu\text{m}^3$.

The sperm head, which has a teardrop shape, with a length of 5–6 μM and a width varying from 2.5–3.5 μM (Sheynkin, 1998), has a volume of 40–50 μm^3 . In other words, sperm head DNA, complexed with protamine according to our structure, will fit very well into the known available space in a sperm head.

7. Discussion

I would like to propose that a structure as logically compelling as this one deserves serious consideration. I would not go so far, however, as to suggest that it be accepted as a complete solution.

In the first place, this is perhaps better described as a “type of structure” rather than a structure *per se*. For example, the inter-residue spacing of 3.4 Å has been selected, although hardly arbitrarily, to match that of the “classic” Watson-Crick structure. Many other spacings are possible within the framework of our basic design. As one extreme example, a structure can easily be made in which the β -sheet is totally extended (i.e. $\phi = \psi = 180^\circ$), which requires a DNA structure which (after intercalation) has a base spacing approximating that of Z-DNA (Wang et al., 1979). Such a structure, however, would not likely be energetically favored under physiological conditions.

Our DNA backbone configuration needs to be optimized. This, however, is an immense undertaking which would be best accomplished by a computer program capable of systematically analyzing millions of possible structures; something I do not have at my disposal.

The structure, as described here, is highly idealized in that it is perfectly linear with respect to the z -axis. In addition to the fact that the sperm cell is obviously not a linear object, there are also such matters as the possibility of *intra*-strand protamine disulfide bonds (Vilfan et al., 2004), or of proline-induced kinks, neither of which I have attempted to deal with, as the existence or non-existence of such things cannot be determined solely by the logical methodology employed herein.

It is worth noting that the essential features of the structure are not ruled out in single-subunit protamine, such as that of the salmon, where there is only one protein subunit type, and no cysteine. Arginine residues projecting from either side of a single β -sheet still allow for the formation of 3 Å salt bridges with “straight ladder” DNA, both in the cross-sectional and axial directions (as per Fig. 2). What this might look like can be readily envisioned by mentally rotating the protein dimers in Fig. 6 (AB, GH, QR and WX) by 90° . The absence of inter-strand protamine cross-links would simply render the nucleoprotein less stable, as has in fact been observed (reviewed in Vilfan et al., 2004).

The suggestion that DNA might, in certain settings, be non-helical, would appear to raise a host of topological

questions. When carefully considered, however, these prove to be far less serious than one might at first presume them to be. These questions have been dealt with elsewhere (Biegeleisen, 2002).

Finally, it may be worth noting that the amino acid sequence of protamine resembles the amino acid sequences of the N-termini and β -bridge regions of the histone octamer, believed to be the binding sites for DNA (Luger et al., 1997a, b). Furthermore, the ratio of positively charged basic amino acid residues to negatively charged DNA phosphate groups is the same in the protamine–DNA complex as it is in the solvent-accessible surface of the nucleosome. The model presented herein may therefore have some implications for structures beyond the sperm head.

Acknowledgments

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References

- Biegeleisen, K., 2002. Topologically non-linked circular duplex DNA. *Bull. Math. Biol.* 64, 589–609.
- Biegeleisen, K., 2005. Protein Data Bank, Theoretical Models section (<http://www.rcsb.org/pdb/search/searchModels.do>), accession numbers 2AWR (Protamine–DNA complex 1) and 2AWS (Protamine–DNA complex 2). (Note: Theoretical models cannot be accessed from the PDB home page).
- Delmonte, C., Mann, L., 2003. Variety in DNA secondary structure. *Curr. Sci.* 85, 1564–1570.
- Luger, K., Mader, A.W., Richmond, R.K., Sargent, D.F., Richmond, T.J., 1997a. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* 389, 251–260.
- Luger, K., Mader, A.W., Richmond, R.K., Sargent, D.F., and Richmond, T.J., 1997b. X-ray structure of the nucleosome core particle at 2.8 Å resolution. *Protein Data Bank* (<http://www.rcsb.org/pdb/>), accession number 1AOI.
- Martins, R.P., Ostermeier, G.C., Krawetz, S.A., 2004. Nuclear matrix interactions at the human protamine domain. *J. Biol. Chem.* 279, 51862–51868.
- Milane, R.P., Rodley, G.A., 1981. Stereochemical details of the side-by-side model for DNA. *Nucl. Acids Res.* 9, 1765–1773.
- Rodley, G.A., Scobie, R.S., Bates, R.H.T., Lewitt, R.M., 1976. A possible conformation for double-stranded polynucleotides. *Proc. Natl Acad. Sci. USA* 73, 2959–2963.
- Sasisekharan, V., Pattabiraman, N., Gupta, G., 1976. *Curr. Sci.* 45, 779–783.
- Sasisekharan, V., Pattabiraman, N., Gupta, G., 1978. Some implications of an alternative structure for DNA. *Proc. Natl Acad. Sci. USA* 75, 4092–4096.

- Sheynkin, Y., 1998. Understanding semen analysis. http://www.uhmc.sunysb.edu/urology/male_infertility/SEMEN_ANALYSIS.html.
- Vilfan, I.D., Conwell, C.C., Hud, N.V., 2004. Formation of native-like mammalian sperm cell chromatin with folded bull protamine. *J. Biol. Chem.* 279, 20088–20095.
- Wang, A.H.J., Quigley, G.J., Kolpak, F.J., Crawford, J.L., Van Boom, J.H., Van Der Marel, G., Rich, A., 1979. Molecular structure of a left-handed double helical DNA fragment at atomic resolution. *Nature* 282, 680–686.
- Wu, T.T., 1969. Secondary structures of DNA. *Proc. Natl Acad. Sci. USA* 63, 400–405.
- Wu, R., Wu, T.T., 1996. A novel intact circular dsDNA supercoil. *Bull. Math. Biol.* 58, 1171–1185.