01.6–5 COMPARISON OF HYDROGEN-DEUTERIUM EXCHANGE IN MYOGLOBIN DERIVATIVES REVEALS LOCALIZED CONFORMATIONAL STABLE REGIONS. By B. P. Schoenborn, N. V. Raghavan and R. M. Fine, Biology Department, Brookhaven National Laboratory, Upton, New York 11973, USA.

Analyses of the H/D exchange ratios for the amide peptides revealed regions within the protein that are not accessible for hydrogen exchange and can be considered as hinge regions for molecular deformations. The occupancy factors of exchangeable hydrogens at amide peptide positions were determined by restraint least square procedures. These reciprocal space refinement techniques (Hendrikson, W. A. and Konnert, J. A. 1980. In: Biomolecular Structure, Vol. 1, pp. 43-57, R. Srinivason, ed. Pergamon, Oxford) were preceded by an analysis of the solvent contribution to the low order reflections (Raghavan, N. V. and Schoenborn, B. P. In: Neutrons in Biology, pp. 247-260, B. P. Schoenborn, ed. Plenum). In the solvent refinement, data from crystals soaked in H_2O and D_2O were used. In order to limit errors in Fourier maps derived from data with often weak intensities with large σ, particular attention to data reduction was given (Schoenborn, B. P. 1983. Acta Cryst. A39: 315-321). The observed change of H/D exchange ratios as a function of soaking time was correlated and compared to local temperature factors. The observed exchange patterns were then analyzed by theoretical dynamical model calculations as developed by C. Levinthal and colleagues. For calculational simplicity, these calculations were restricted to particularly interesting regions like the GH corner.

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01.6–6 THE CRYSTAL STRUCTURE OF THE NUCLEOSOME CORE PARTICLE BY NEUTRON DIFFRACTION. By G.Bentley(1), A.Lewit-Bentley(1),J.T.Finch(2),A.D.Podjarny(3) and M.Roth. (1)E.M.B.L.,Grenoble,(2)M.R.C.,Cambridge,U.K., (3)N.I.H.,Bethesda,U.S.A.,(4)I.L.L.,Grenoble,FRANCE.

The crystal structure of the Nucleosome has been studied with neutrons to 16Å resolution. By using H_2O/D_2O solvent contrast variation, the structures of the DNA (146 base pairs) and the protein (eight histone subunits) components could be analysed separately. The histone core was solved at the 65% D_2O contrast (DNA match point) and refined by density modification. The protein density, though roughly helical, is broken up into four regions of about equal volume and we interpret these as being dimers of two kinds: (H2A-H2B) and (H3-H4). Because solvent contrast variation can distinguish between hydrophobic and hydrophilic regions of protein density, the results suggest that the interface between the monomers in each histone dimer is hydrophobic in character while the interactions between dimers in the histone octomer are weaker and/or more hydrophilic in character (hydrophilic regions are weak in contrast with respect to 65% D_2O solvent). The DNA structure was solved at 39% D_2O, the match point of the protein. Its structure was refined as a super-helix by the structure factor least-squares procedure. The refined super-helical parameters give a pitch of 25.8±0.4Å, a radius of 42.1±0.2Å and 1.81±0.01 turns of DNA. The effect of the uneven scattering from the protein (hydrophobic and hydrophilic regions) had to be accounted for in the least-squares refinement of the DNA. Most of the DNA is in contact with the protein. Although the nucleosome possesses a non-crystallographic dyad, departures from this can be seen in the relation between the two (H2A-H2B) dimers.

01.7–1 ESTIMATES OF LATTICE PARAMETERS AND SYMMETRY OF PROTEIN CRYSTALS BY ELECTRON MICROSCOPY. By R. H. Lange, Department of Anatomy and Cell Biology, University of Giessen, Aulweg 123, D-6300 Giessen.

Based on the analysis by electron microscopy (EM) of 7 protein crystal preparations, for which comparative X-ray data existed, we conclude that EM of fixed, embedded, thin-sectioned and heavy-metal stained (and stabilized) protein crystals provides crystal data quite close to that from X-ray analysis (Table I). Heavy-metal treatment of fixed protein crystals enhances electron diffraction rather than alters the character of the lattice (Fig. 1). Problems of the EM approach are: deformation of the lattice during specimen processing, shortcomings of the electron microscope as a measuring device, limited resolution. To cope optimally with these prob-

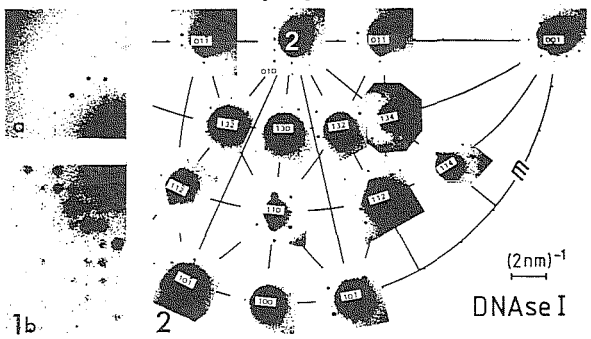

Fig. 1. Lipovitellin-phosvitin similar to Table I, 5-8 (L. chalumnae: Lange, C. R. Acad. Sci. III (1983) 297, 393) [100] (a: no heavy metal, b: heavy metal treatment).
Fig. 2. Reciprocal lattice reconstructed using intersecting series of electron diffraction patterns mounted in a stereographic projection (Table I, 14).

Table I: Comparative X-ray (X) and EM *(EM)* data of protein crystals ([1]single crystals, [2]powder, *in-vivo crystals)

Specimen	Technic, Resolution [nm], Symmetry	a	b	c	β	Ref.
		[nm,°], (rounded)				
1: GLUCAGON, pig	X^1; 0.3, $P2_13$	4.7				1
2: -, teleost*	EM^1; *2.4, $P2_13$ poss.*	*4.1-4.8*				2
3: 2.5% Zn-INSULIN	X^2 } trigonal	8.2		3.3		3
4: -, 30% hydrated	EM^1; *0.8* } (R)	*7.4*		*3.1*		
LIPOVITELLIN-PHOSVITIN, *Xenopus laevis*						
5: dried*	X^2; 2.5, 222	7.8	15.8	17.6		4
6: dehydrated*	EM^1; *1.9, $P2_1[2_12_1]$*	*8.4*	*16.0*	*18.1*		5
7: wet*	X^2; 2.5,222*($P2_122_1$)*	8.9	17.2	19.6		6
8: 30 % hydrated*	EM^1; *1.6, $P2_1[2_12_1]$*	*9.1*	*17.6*	*19.2*		5
-, *Ichthyomyzon unicuspis*						
9: wet	X^1; 0.3, C2	19.3	8.8	9.2	101.3	7
10:30 % hydrated	EM^1; *3.0, C2*	*19.4*	*8.9*	*8.9*	*103.4*	8
-, *Petromyzon marinus*						
11:wet	X^1; 0.3, C2	19.3	8.8	9.1	101.3	7
12:dehydrated*	EM^1; *2.5, C2*	*20.1*	*9.1*	*9.3*	*103.1*	8
13:DNAse I, wet	X^1; 0.25, C2	13.2	5.5	3.8	91.4	9
14:-, 30% hydrated	EM^1; *2.0, C2*	*12.2*	*5.2*	*4.0*	*96.4*	10

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lems, the EM method has been standardized (Lange, J. Ultrastruct. Res. (1982) 79, 1). It involves combined specimen tilting (± 60°, Philips EM 201, 400) and selected area diffraction (50-400 diffraction patterns for a consistent model of the reciprocal lattice, Fig. 2). The EM approach is the sole method for studying natural protein crystals in situ; it is particularly suitable for large unit cells (Table I, 5-12). In the case of smaller unit cells (Table I, 14) quasi-dynamic effects are frequent and more diffraction patterns are required. Despite its limited accuracy the EM approach has proved indispensable and encouraging.