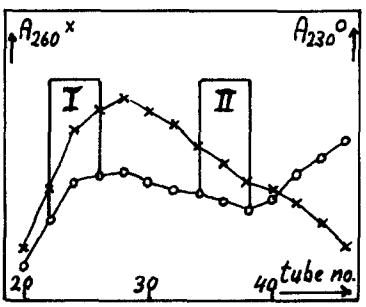
Structural studies on fractionated higher order chromatin by measurement of small-angle X-ray scattering (SAXS), sedimentation, viscosity, and quasi-elastic light scattering (QLS)

R. Brust, B. Meuel, H. Notbohm

Institut f. Med. Molekularbiologie d. Medizinischen Hochschule Lübeck, Ratzeburger Allee 160, 2400 Lübeck, G.F.R.

Soluble rat liver chromatin was prepared by autolyzing isolated nuclei and fractionated by gel chromatography on Sepharose 2B (see Fig.). Main component of all chromatin containing tubes (no. 20-40) is quaternary structure chromatin which can be approximated by a solenoidal model with an outer diameter of 32-34 nm. According to SAXS studies we find an increasing content of tertiary structure chromatin - i.g. nucleosomal chains - with increasing tube numbers. The ratio of the relative amount of tertiary structure in pool I to that in pool II is 0.5 (1).



Determination of the s-values by sedimentation velocity measurements, of the diffusion coefficient D by QLS and of the viscosity η by measurement with a new kind of a diver-rotation-viscometer gives additional information about chromatin in pool I and pool II:

	s (S)	D(10^{-7} cm^2 sec^{-1})	η (g·dl^{-1})	Mx10^{-6}	βx10^{-6}
I	130-200	0.4-0.7	0.2-1.2	16-36	3.8-2.2
II	65-90	0.7-1.0	0.1-0.4	5-10	3.1-2.2

Molecular weights M as calculated from s and D are in good agreement with those deduced solely from s-values according to Butler & Thomas (2). Due to the parameters s, D and η different contents of tertiary structure chromatin in pool I and II are not detectable. This corresponds merely to SAXS data asssuming the content of tertiary structure being very low both in pool I and II. Thus, the experimental determination of s, D and η has to be refined considerably for evaluating these slight structural differences.

A certain hint may be the factor β in the Mandelkern-Flory-equation. β seems to be slightly larger for pool I as compared to pool II, when calculated from s, η and M according to the chromatin data. An additional indication for this structural difference is evident from biochemical studies on chromatin function: kinetic experiments on the replication pattern of rat liver chromatin after partial hepatectomy show that chromatin in pool II is preferentially replicated in the early S-phase, whereas pool I chromatin with lower content of tertiary structure chromatin is mainly replicated in the later S-phase (3).

1. Brust,R. and Harbers,E.(1981), Eur. J. Biochem., in press.
2. Butler, P.J.G. and Thomas, J.O. (1980), J. Mol. Biol. 140, 505-529.
3. Brust,R. and Harbers,E.(1981), Biochem. Biophys. Res. Comm., in press.