J. Membrane Biol. 34, 93–101 (1977)

The Molecular Configuration of Inulin: Implications for Ultrafiltration Theory and Glomerular Permeability

E. Middleton

Department of Hormone Physiology, Imperial Cancer Research Fund, London, WC2A3PX, England

Received 2 November 1976

Summary. A space-filling model of the inulin molecule, based solely on physico-chemical data in the literature, indicates that the gross shape of the solvated molecule is a cylinder having semi-length 25 Å and radius 10 Å. The axial dimensions of this model very nearly equal the equivalent estimates (25 and 10.5 Å) previously determined by studying restricted passage of inulin through artificial membranes of known pore size (E. Middleton, *J. Membrane Biol.* **20**:347, 1975). The good agreement between these independent *in-vitro* studies—neither of which depends on ultrafiltration theory—strongly suggests that glomerular pore size is considerably greater than predicted by the theory.

Despite the almost universal use of inulin clearance as a measure of glomerular filtration (Materson, 1971; Bianchi, 1972; Renkin & Gilmore, 1973; Lassiter, 1975), the actual dimensions of the inulin molecule and of the glomerular pores have not been—and in the latter case, probably cannot be—measured directly. In a critical article on the viscosity of macromolecules, Yang (1961) makes it abundantly clear that no single method suffices to establish molecular configuration beyond doubt. Cross-matching of results using different methods is therefore necessary. The present paper considers the shape and dimensions of the inulin molecule which best fit its known physico-chemical properties, and discusses how the findings concern ultrafiltration theory and glomerular permeability.

The Inulin Molecule

Inulin is a polysaccharide consisting mainly of fructofuranose sub-units. The main feature of this sub-unit (Fig. 1) is an angular carbon 'ring' centered on an oxygen atom; the other constituent particles can

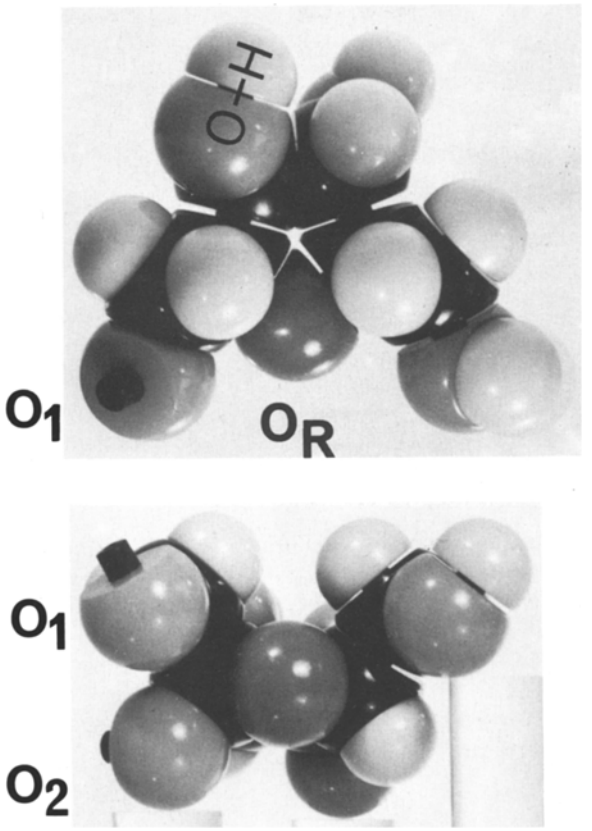


Fig. 1. Space-filling views of the fructofuranose unit. The ring-oxygen (O_R), link-oxygens (O_1, O_2), and the 3-position hydroxyl group (O–H) are identified. Carbon: dark; hydrogen: light; oxygen: medium

be rotated geometrically, though possibly not chemically, about the ring, but are shown in the symmetrical position. In particular, the two key oxygen atoms (O_1, O_2) available for linking units together (seen at the left side of the carbon ring) are shown square-on to the ring. The present model indicates overall dimensions for the fructofuranose unit of about $10 \times 9 \times 5$ Å. Phelps (1965) gives the dimensions as $12 \times 3.5 \times 3$ Å, but since he gives no diagram of the unit we may have misinterpreted what he says.

The average inulin molecule consists of 30 fructofuranose units linked together in a nonbranching chain which is terminated at one end by a nonreducible D-glucose unit (Hirst, Mcgilvray & Percival, 1950; West, Todd, Mason & Van Druggen, 1966; Aspinall, Percival, Rees & Rennie, 1967). This structure has a molecular weight of about 5000. Since the

fructofuranose units are (2→1) linked (Lindberg, Lonngren & Thompson, 1973), the resulting configuration is essentially compact rather than extended. Phelps (1965) suggests that the known physical data on the inulin molecule points to its configuration being a helix of four fructofuranose units per turn.

A helix of *exactly* four fructofuranose units per turn is not possible however, since the nth unit would collide with the $(n+4)$th unit. This arises from the fact that, for such a helix (link-oxygens square-on to their adjacent carbons; carbon rings parallel to one another and orthogonal to the length of the helix), there would be a rise per unit of 0.91 Å and a total rise in four units of 3.6 Å, which is considerably less than the overall height of one unit (5 Å).

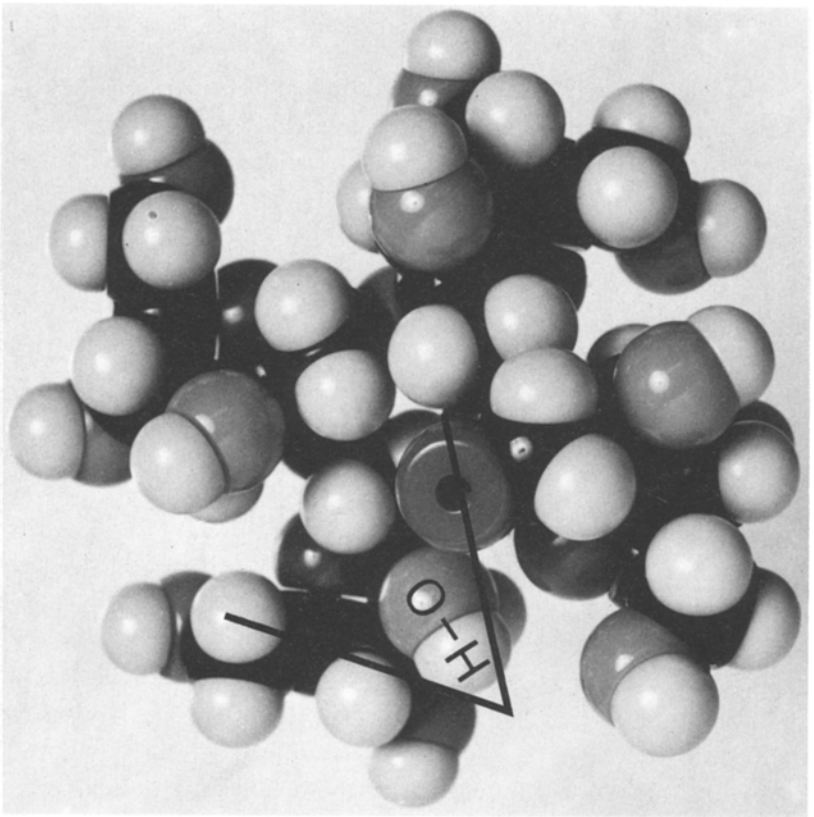
Slight orientation of the link-oxygens in a clockwise direction (in the sense of Fig. 1) allows the rise per unit to be increased to the point where collision between units is avoided. We find that the resulting shortest helix has semi-length 18.5 Å and radius 8 Å. By orientating the link-oxygens still further, the length of the helix can be further increased, though the number of units per turn and the radius of the helix will be reduced.

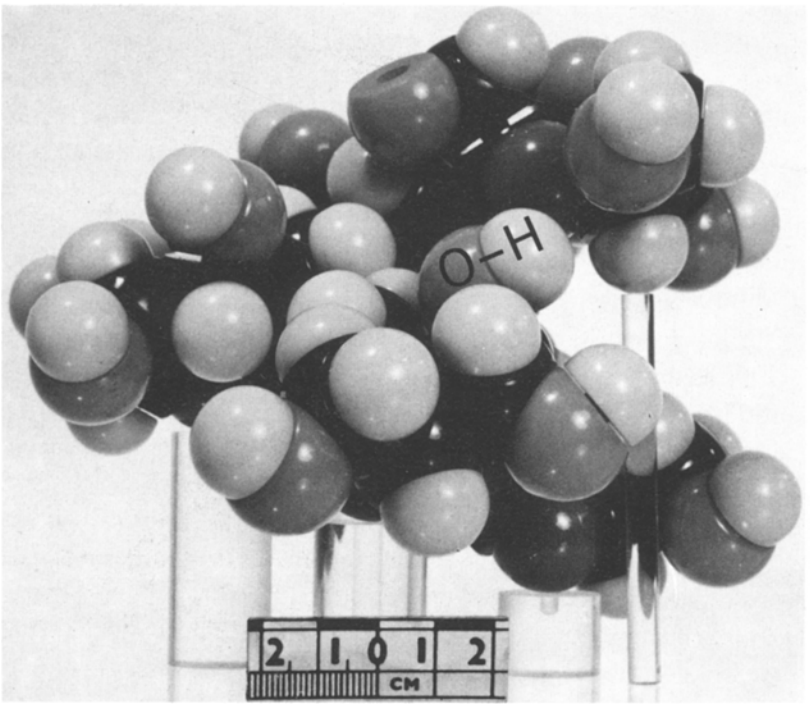
Assuming that an estimate of four units per turn is nearly correct, and that there is no bonding between atoms in adjacent turns, then the exact number of turns will be determined by the Van der Waals radii of those atoms which, though geometrically capable of touching each other, would in fact be separated by some minimum distance (hydrogen: 1.2 Å; oxygen: 1.4 Å) (Lange, 1973). The relevant atoms here are (Fig. 1) the ring-oxygen (O_R), which is the central feature of the lower surface of the unit, and the hydroxyl group in the 3-position (O–H), which is the most prominent feature of the upper surface.

By comparing the relative positions of unit 6 (skeletal display) and unit 2 (full display) in Fig. 2 (upper) it can be seen that O_R in the $(n+4)$th unit lies close to, and slightly above, the 3-position O–H group in the nth position. The situation can be visualized in elevation by imagining unit 6 placed in position above unit 2 in Fig. 2 (lower). On compressing the helix, it unwinds to the point where the atoms in question collide.

From this we find that the 'Van der Waals' helix (Fig. 2) has about 7.7 turns (3.9 units per turn); semilength 22.5 Å; radius 7.5 Å; pitch 6 Å and, since the overall height of the fructofuranose unit is about 5 Å, a separation of about 1 Å between turns.

The present model indicates that when more than four fructofuranose units are linked together, the bond angle between the relevant link-atoms


3
4
2
5
O-H


5
3
O-H
1
2 1 0 1 2
CM

is increased. Physicochemical constraints will place an upper limit on this increase which itself may not be constant along the length of the helix; in this case, the increase will be maximal at the center of the helix. We note that a slight decrease in bond angle from the center to the extremities of the helix could bring the last two adjacent turns into contact with each other at each extremity, thereby limiting the helix to a certain number of fructofuranose units (30 units per average inulin molecule). This would not noticeably affect the overall dimensions of the macromolecule.

This helical model for the inulin molecule refers to the unsolvated molecule whereas, in practice, we are concerned with the solvated molecule. Horowitz and Moore (1974) have studied the movement of ^{3}H-inulin microinjected into the cytoplasm of oocytes of *Rana pipiens,* and one of their conclusions was that water associated with the macromolecular matrix was not appreciably modified from the free solution. This seems to imply that the water was not tightly bound.

In the absence of evidence to the contrary, it seems reasonable to assume that external hydration of the inulin molecule is limited to a monolayer of attracted water molecules. It also seems reasonable to assume that internal hydration does not determine the length of the molecule, since the increase in bond angle between the link-atoms of the inulin molecule (necessary for forming the helix) is probably near the maximum permissible without the bond breaking. We might therefore expect solvation to increase the dimensions of the inulin molecule from $22.5 \times 7.5 \times 7.5$ Å to $25 \times 10 \times 10$ Å (the diameter of the water molecule is about 2.5 Å).

Discussion

The good agreement between the present estimate of the size of the inulin molecule (semi-axes 25 and 10 Å) based solely on physicochemical data in the literature, and a previous estimate (semi-axes

Fig. 2. Space-filling views of one turn of the present model for the inulin molecule. Each turn consists of about four fructofuranose units. The 3-position O–H group of unit 2 is identified in both views to aid description in text. Scale (approx) 1 cm = 1 Å. *Upper view*: This shows four full units and one skeletal unit. The helix travels clockwise out of the plane of the paper. *Lower view*: This uses five units to show the full pitch of the helix. The fifth unit lies almost directly above the first; the 3-position O–H group of unit 1 is out of view

25 and 10.5 Å) determined by studying restricted passage of the solute through artificial membranes of known pore size (Middleton, 1975), suggests that the present estimate of configuration is substantially correct. Although these estimates do not depend on ultrafiltration theory, we shall discuss them in terms of the theory, particularly where this concerns glomerular permeability.

Ultrafiltration theory defines the relation between membrane sieving of a solute and membrane pore size in terms of the Stokes-Einstein radius of the molecule, and is often used to estimate membrane pore size (Landis & Pappenheimer, 1963; Renkin & Gilmore, 1973; Chang, Ueki, Troy, Deen, Robertson & Brenner, 1975). Whether such estimates are valid when the marker molecule is not spherical is open to question, since the Stokes-Einstein radius is simply the effective *imaginary* radius of the molecule treated as though is were a sphere, measured in the special circumstances of free diffusion. It seems logical to assume that sieving equations lead to *imaginary* 'Stokes-Einstein' pore sizes when the Stokes-Einstein radius of a nonspherical molecule is used to measure permeability. Perhaps we should note that sieving equations are basically about kinetics rather than molecular and pore sizes, and the kinetics are not invalidated simply because these sizes are imaginary.

Confidence in ultrafiltration theory is apparently not absolute, even when spherical molecules are used. Renkin and Gilmore (1973) point out that "theory is based on crude macroscopic models of restriction". Bean (1972) finds that "theory for fine pores – where pore and molecular sizes are comparable – is somewhat underdeveloped". Chang *et al.* (1975) state that "convincing evidence for the validity of a hydrodynamic model of porous membrane transport is still lacking".

The situation is even less satisfactory where nonspherical molecules are concerned. Renkin and Gilmore (1973) suggest that, where clearance of substances of identical molecular weight are unequal, the differences are probably accounted for by differences in molecular configuration. Ackers and Steere (1967) and Kagawa (1974) suggest that molecular configuration affects molecular weight determinations in gel filtration studies. Of great practical importance here is the recent finding by Nozaki, Schechter, Reynolds and Tanford (1976) that the Stokes-Einstein radius of a large asymmetric protein particle estimated using gel chromatography, was considerably lower than its true Stokes-Einstein radius measured by hydrodynamic methods.

Their interpretation of this phenomenon is that "end-on insertion of asymmetric particles into gel pores contributes to their retardation"

(down the gel). In view of this, it is worth comparing the situation for a prolate particle (length > diameter) with that for an oblate particle (diameter > length) having the same Stokes-Einstein radius.

Although both particles, and the hydrodynamically equivalent sphere, diffuse at equal rates in free solution, the prolate particle can pass through a smaller pore than can the equivalent sphere, which in turn can pass through a smaller pore than can the oblate particle. In other words, gel chromatography might be expected to underestimate the Stokes-Einstein radius of a prolate particle, and overestimate it for an oblate particle. Clearly, particle configuration, as well as size, must be taken into account in filtration studies.

According to Wesson (1969) passage of a solute through a membrane starts to be restricted when the radius of the (spherical) molecule exceeds 25% of the pore radius. This is implicit from the classic plot of filtration fraction (F/P) against molecular radius (a_e) (Landis & Pappenheimer, 1963; Renkin & Gilmore, 1973). For prolate molecules it seems likely that hindrance commences when the major semi-axis equals 25% of the pore radius, and is complete when the minor semi-axis equals the pore radius. The first situation is governed by those molecules presented with their long axis parallel to the plane of the pore, since these appear to the pore as large spheres; the second is governed by those molecules presented with their minor axis parallel to the pore, since these appear as small spheres. We assumed this to be so for the purpose of determining the axes of the inulin molecule from sieving experiments, and it is of interest to consider how this reasoning affects estimation of glomerular pore size.

Micropuncture study of glomerular fluids has shown beyond doubt that inulin filters freely through glomerular membranes in amphibia (Hendrix, Westfall & Richards, 1936; Bott, 1952; Giebisch, 1956) and in the Munich-Wistar rat (Harris, Baer, Chirito & Dirks, 1974; Chang *et al.*, 1975). Sieving experiments have indicated that passage of inulin starts to be restricted at a pore radius of 100 Å, irrespective of the mode of transport (Middleton, 1975). Taken together, these findings suggest that glomerular pore radius is at least 100 Å, though Renkin and Gilmore (1973) have estimated from ultrafiltration theory and the Stokes-Einstein radius of various proteins (including serum albumin) that it is about 34 Å.

The importance of serum albumin lies in its size, since it separates those molecules which can filter through the glomerular membranes from those which cannot. The filtration end-point for serum albumin is a

pore having radius 55 Å (Jacobs, 1974), and this represents either the minor axis of a prolate form, or the major axis of an oblate form. The Stokes-Einstein radius of the molecule is 36 Å (Renkin & Gilmore, 1973) and, since this radius must lie between the minor and major axes, the molecule would appear to be oblate having radius 55 Å and semi-length roughly 17 Å (axial ratio 3.2). Yang (1961) questions whether the choice of a prolate model is correct, and points out that X-ray scattering studies suggest an oblate model having an axial ratio of 3.5.

Whether a molecule is prolate or oblate is not a trivial matter when using its Stokes-Einstein radius to estimate glomerular permeability, since the two forms have completely different filterabilities. Moreover, the manner in which hindrance increases as pore size decreases is also very different for the two forms. It would be very difficult to write ultrafiltration theory to accommodate these factors simultaneously.

In conclusion, if suitably large spherical molecules could be manufactured for use in conjunction with ultrafiltration theory, they might predict a completely different glomerular pore size than is obtained using non-spherical molecules.

I would like to thank Dr. J.A. Wright for helpful discussions about the inulin molecule and Mr. G.D. Leach AIIP for photographing the model.

References

Ackers, G.K., Steere, R.L. 1967. Molecular sieve methods. *In:* Methods in Virology. K. Maramorosch and H. Koprowski, editors. p. 341. Academic Press Inc., New York & London

Aspinall, G.O., Percival, E., Rees, D.A., Rennie, M. 1967. Oligosaccharides, polysaccharides and related compounds. *In:* Rodd's Chemistry of Carbon Compounds. S. Coffey, editor. p. 687. Elsevier Publishing Company, Amsterdam, London & New York

Bean, C.P. 1972. The physics of porous membranes—neutral pores. *In:* Membranes. Vol. 1, Macroscopic Systems and Models. G. Eisenman, editor. Marcel Dekker, New York

Bianchi, C. 1972. Measurement of glomerular filtration rate. *Prog. Nucl. Med.* **2**:21

Bott, P.A. 1952. Renal excretion of creatinine in *Necturus*. A reinvestigation by the direct analysis of glomerular and tubular fluid for creatinine and inulin. *Am. J. Physiol.* **168**:107

Chang, R.L.S., Ueki, I.F., Troy, J.L., Deen, W.M., Robertson, C.R., Brenner, B.M. 1975. Permselectivity of the glomerular capillary wall to macromolecules. II. Experimental studies in rats using neutral dextran. *Biophys. J.* **15**:887

Giebisch, G. 1956. Measurement of pH, chloride and inulin concentration in proximal tubule fluid of *Necturus*. *Am. J. Physiol.* **185**:171

Harris, C.A., Baer, P.G., Chirito, E., Dirks, J.H. 1974. Composition of mammalian glomerular filtrate. *Am. J. Physiol.* **227**:972

Hendrix, J.P., Westfall, B.P., Richards, A.N. 1936. Quantitative studies of the composition of glomerular urine. XIV. The glomerular excretion of inulin in frogs and *Necturi*. *J. Biol. Chem.* **116**:735

Hirst, E.L., Mcgilvray, D.I., Percival, E.G.V. 1950. Studies of fructosans. Part I. Inulin from dahlia tubers. *J. Chem. Soc.* p. 1297

Horowitz, S.B., Moore, L.C. 1974. The nuclear permeability, intracellular distribution, and diffusion of inulin in the amphibian oocyte. *J. Cell Biol.* **60**:405

Jacobs, S. 1974. Ultrafilter membranes in biochemistry. *In:* Methods of Biochemical Analysis. D. Glick, editor, p. 330. John Wiley & Sons, New York, London, Syndney & Toronto

Kagawa, Y. 1974. Dissociation and reassembly of the inner mitochondrial membrane. *In:* Methods in Membrane Biology. E.D. Korn, editor. p. 224. Plenum Press, New York & London

Landis, E.M., Pappenheimer, J.R. 1963. Exchange of substances through the capillary walls. *In:* Handbook of Physiology. W.F. Hamilton and P. Dow, editors. Sect. 2, Circulation. American Physiological Society, Washington, D.C.

Lange, N.A. 1973. Atomic and molecular structure. *In:* Lange's Handbook of Chemistry. J.A. Dean, editor. Ch. 3, p. 120. McGraw-Hill, New York

Lassiter, W.E. 1975. Kidney. *Annu. Rev. Physiol.* **37**:371

Lindberg, B., Lonngren, J., Thompson, J.L. 1973. Methylation studies on levans. *Acta Chem. Scand.* **27**:1819

Materson, B.J. 1971. Measurement of glomerular filtration rate. CRC Critical Reviews in Clinical Laboratory Sciences. p. 1

Middleton, E. 1975. Passage of inulin and *p*-aminohippuric acid through artificial membranes: Implications for measurement of renal function. *J. Membrane Biol.* **20**:347

Nozaki, Y., Schechter, N.M., Reynolds, J.A., Tanford, C. 1976. Use of gel chromatography for the determination of the Stokes radii of proteins in the presence and absence of detergents. A reexamination. *Biochemistry* **15**:3884

Phelps, C.F. 1965. The physical properties of inulin solutions. *Biochem. J.* **95**:41

Renkin, E.M., Gilmore, J.P. 1973. Glomerular filtration. *In:* Handbook of Physiology. J. Orloff and R.W. Berliner, editors. Sect. 8, Renal Physiology. American Physiological Society, Washington, D.C.

Wesson, L.G. 1969. Physiology of the Human Kidney. pp. 82–93. Grune & Stratton, New York & London

West, E.S., Todd, W.R., Mason, H.S., Van Druggen, J.T. 1966. Carbohydrates, or the saccharides. *In:* Textbook of Biochemistry. p. 246. Macmillan, New York; Collier-Macmillan, London

Yang, J.T. 1961. The viscosity of macromolecules in relation to molecular conformation. *In:* Advances in Protein Chemistry. C.B. Anfinsen, M.L. Anson, K. Bailey and J.T. Edsall, editors. p. 323. Academic Press, New York & London