TRANSCORTIN: A NEUTRON SCATTERING STUDY OF A GLYCOPROTEIN

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1. Introduction

The structure of serum glycoproteins is not known to the same extent as that of non-glycosylated proteins. Very few of them have been crystallised, and with the exception of immunoglobulin fragments [1], only low resolution X-ray diffraction data has been reported [2]. Such low resolution X-ray data reveal very little about the relative organisation of the sugar and protein constituents. This structural information must be obtained by more indirect techniques. Among methods providing low resolution structure, neutron scattering has been proved to be specially useful for particles made of two different chemical components, e.g., proteins and nucleic acid or proteins and lipids [2–6]. The introduction of D_2O in the solvent is used to change the contrast between that solvent and the various components, and it is for different amounts of D_2O that the scattering density of protein and of nucleic acid or lipid is matched by that of the solvent. For these 3 types of compound the respective percentages of D_2O are 40%, 65–70%, and 10%. It was not a priori obvious that this method could be applied to glycoproteins; indeed in a pure water solvent (no D_2O) the scattering density of protein and sugar are expected to be nearly identical. We show here, however, that those two components scatter very differently in a D_2O solvent; therefore, they can be distinguished and consequently structural information can be obtained on the two moieties of a glycoprotein.

Here, the glycoprotein studied is the human transcortin or corticosteroid binding globulin (CBG), which specifically binds corticosteroid hormones in the blood [8]. Its M_r value has been estimated from measurements of the sedimentation and diffusion coefficients to be ~50 000 [9,10]. It consists of a single polypeptide chain and several osidic chains accounting for 27% (w/w) of the molecule [9] and has, to our knowledge, never been obtained in a crystalline state. Neutron scattering was therefore considered with the double aim to test its usefulness with glycoproteins and if possible to obtain some structural information.

2. Materials and methods

Transcortin was isolated from human blood and purified as in [11] by affinity chromatography. It was stored as a lyophylised powder. Prior to experiments the protein was dissolved in a buffer Tris 50 mM, NaCl 200 mM (pH 8.3) then dialysed against the same buffer containing 7 mg/l cortisol to stabilize it. The water of the buffer was a mixture of normal and heavy water. After a minimum of 24 h dialysis, samples were centrifuged at low speed to eliminate aggregates. The concentration was measured by UV transmission using an absorbance of 0.707 for 1 cm at 280 nm. This was done directly in the same quartz cells used for neutron experiments (optical path 0.1 and 0.2 cm). The neutron scattering method has been described [3] and will be summarized in section 3.

Data were collected under the following conditions: wavelength 8 Å; sample to detector distance 255 cm; D_2O percentage in the buffer, 0%, 25%, 62%, 75%, 96.5% and 100%. These percentages were checked by neutron transmission [3]. With these conditions only the central part of the scattering pattern would be measured. This is enough to determine two relevant parameters: the intensities extrapolated to zero scattering angle and the radius of gyration both as a function of the D_2O percentage in the buffer.

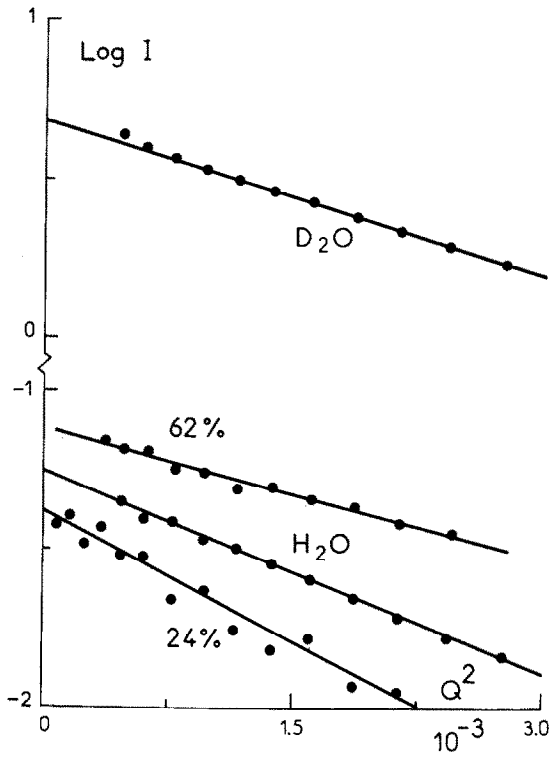

Fig.1. A plot of the logarithm of the scattered intensities versus Q^2. Q the scattering vector is related to the scattering angle θ and the neutron wavelength λ by: $Q = (4\pi/\lambda) \sin(\theta/2)$. Each set of points is for a given amount of D_2O in the buffer.

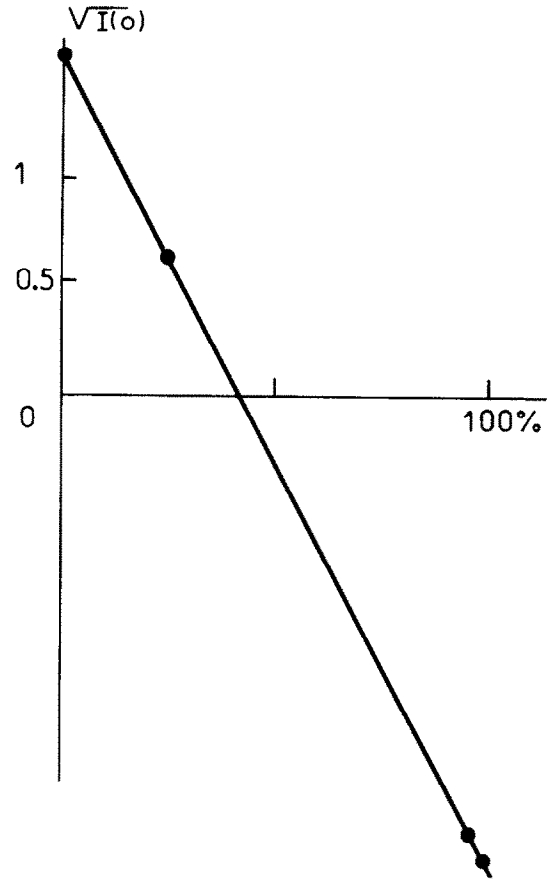

Fig.2. Square root of the intensity scattered at zero angle versus the percentage of D_2O in the solvent. Intensities have been corrected for the transmissions, thicknesses and concentrations of the samples.

3. Results

Fig.1 shows the scattering curves obtained in various contrasts. There is absolutely no indication of aggregation. This is rather remarkable, as normal proteins when lyophylised tend to show strong aggregations when put into solution. The other noticeable feature is the very large angular range over which holds the Guinier approximation:

$$I = I(O) \exp(-\frac{4\pi^2}{3} \frac{\theta^2}{\lambda^2} R_G^2)$$

where I is the intensity scattered at the angle θ, λ the neutron wavelength and R_G the radius of gyration. This suggests that in all the solvents the transcortin looks quite like an ellipsoid with a small axial ratio.

3.1. *The intensity at the origin and the molecular weight*

Fig.2 shows the square root of the normalized intensity at the origin versus the D_2O content. For a monodisperse system, this plot must be linear. The intensity vanishes for a D_2O percentage such that:

$$\Sigma b - b_s V = 0$$

Σb is the sum of the scattering amplitude over all the atoms of the molecule. For neutrons, deuterium scatters differently from normal hydrogen, thus the scattering power of the molecule varies with the D_2O percentage in the solvent. b_s is the scattering density of the solvent, and is simply given as a function of the percentage X of D_2O by:

$$b_s = (-0.562 + 6.96X)\ 10^{-14}\ \text{cm/Å}^3$$

V is the volume occupied by the atoms of the molecules.

The intensity $I(O)$ in water can be used to estimate the M_r [3,12]. In water, sugars and proteins have the same value of $\Sigma b/V$, the scattering density, and consequently the same contrast with the H_2O solvent. The relation of proportionality between the intensity $I(O)$ and the M_r of a protein [10] must simply be modified to take into account the difference between the specific volume of sugar and protein:

$$I(O) \approx (M_P + M_S)\left(1 - \frac{\bar{\nu}_P - \bar{\nu}_S}{\bar{\nu}_P}\,\frac{M_S}{M_S + M_P}\right)^2$$

where the subscript P is for protein and S for sugar. To use this formula one needs the weight ratio of the two moieties and their respective specific volumes. We have used the weight ratio of 27% of sugar in [9]. For sugar a specific volume of 0.60 cm^3/g was assumed. This value is based on two estimations: (i) based on an extrapolation of the published specific volume of glycoproteins of various sugar content to 100% sugar, giving $\bar{\nu} = 0.59$ (with a large uncertainty); (ii) using the densities of sucrose solution, giving $\bar{\nu} = 0.62$. For the protein, $\bar{\nu} = 0.75$ was assumed. This leads to M_r 52 000 in good agreement with the values deduced from sedimentation and diffusion, namely 51 700 [10] and 49 500 [9]. Now one can use this M_r value together with the chemical composition in [9] to calculate $(\Sigma b - b_s V)$ in various solvents. To do this we assume that 80% of the exchangeable protons of the protein and 100% of those of the sugar do exchange. The scattering amplitude of the amino acid, the exchangeable protons and the volume of amino acid were those given in [3]. The values in table 1 are deduced. With those values one finds that the scattering of the protein vanishes for 38.9% D_2O, that of the sugars for 56.6% and that of the transcortin for 42.1%. The experimental value of 42% agrees well with that prediction.

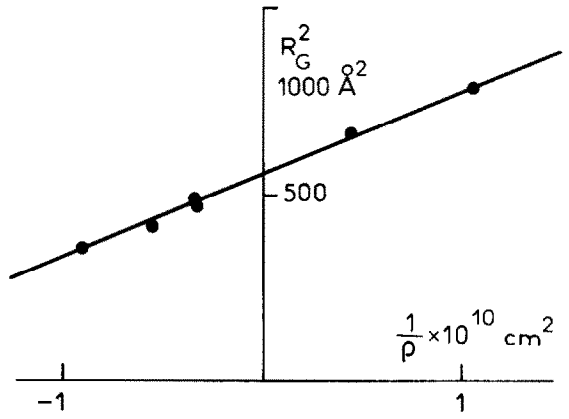

Fig.3. Variations of the square of the radius of gyration with the inverse of the contrast. The contrast is defined as the difference between the scattering density of the solute and that of the solvent. The linearity of the plot proves that the center of gravity of the sugars coincides with that of the protein.

3.2. *The radius of gyration*

Fig.3 shows the variation of the square of the radius of gyration R_G with the inverse of the contrast. In this Stuhrmann representation [13], a linear variation indicates that the centers of gravity of the protein and of the sugars coincide [3]. For a uniform distribution of scattering matter, the radius of gyration should be contrast-independant. Here one sees that R_G varies very strongly with contrast, 4-times more than with a normal globular protein. (For a protein the ratio R_G in H_2O/R_G in D_2O is between 1.01 and 1.05. For transcortin this ratio is 1.18.) This strong variation is due to the sugar. At some contrasts the scattering is dominated by the protein part which is a much smaller object than the whole molecule with its sugar. This analysis can be put into quantitative terms.

For a protein of M_r 34 000 the R_G is expected to be ~19.0 Å. This is the value found for transcortin at

Table 1

	M_r	V	Σb(in H_2O)	Exchangeable protons	$\Sigma b - b_s V$
Sugar	14 040	14 040	$263 \cdot 10^{-12}$ cm	414	$(347-613X)\ 10^{-12}$ cm
Protein	37 960	47 450	$825 \cdot 10^{-12}$ cm	543	$(1087-2792X)\ 10^{-12}$ cm
Complete molecule	52 000	61 490	$1088 \cdot 10^{-12}$ cm	957	$(1434-3405X)\ 10^{-12}$ cm

X is the percentage of D_2O in the water of the buffer

~60% D_2O, in fair agreement with the estimation that the sugars are density matched by solvent with 57% D_2O. One can then use this value and the relation:

$$R^2_{G,H_2O} = R^2_{G,P}\ f_P + R^2_{G,S}\ f_S$$

where f is the relative weight of the scattering power:

$$f_P = \frac{0.73 \times 0.39}{(0.73 \times 0.39) + (0.27 \times 0.57)}$$

Using the experimental value for R_G in H_2O (26 Å), one gets for R_G sugar 35.5 Å. To get a better feeling of the implications of these numbers, one can consider transcortin as a spherical particule. It would then have a protein core of 24.5 Å radius (R_G 19 Å) with a sugar shell extending from 24.5–42.5 Å. The transcortin is certainly not a sphere, but it is certainly, as noticed above, close to an ellipsoid with a small axial ratio, and the thickness of the sugar is certainly close to the 18 Å found for the spherical model. The fact that the scattering curves are rather similar at various contrasts supports the idea of a shape which is contrast-independant, which implies a rather uniform distribution of the sugar on the surface of the protein.

4. Conclusions

Clearly neutron scattering can be used to analyse a glycoprotein with a large amount of sugar. The data support a large difference between the solvent composition which matches the scattering densities of the protein and of the sugars. Transcortin appears as a slightly elongated ellipsoid with a sugar shell 18 Å thick. This thickness, for 12 000 M_r, implies a huge hydration, and transcortin is certainly close to the classical image of a protein with long rigid hairs. This model is in fair agreement with the hypothesis concerning the localisation of glycan moiety: it should constitute an 'aerial' zone formed by 'antennae' planted on the protein core [14]. Such a conclusion was also deduced from the predicted secondary structure of the peptide segment around the carbohydrate–peptide linkage [15]. The lack of knowledge about the sugar and amino acid sequences, prevents further analysis, which will be done, in better known glycoprotein such as orosomucoid (α_1-acid glycoprotein).

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