J. Pharm. Pharmac., 1972, 24, 513–517 *Received December* 29, 1971

Structure of an iron-dextran complex*

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An iron-dextran complex* from which most of the uncomplexed dextran present had been removed by ultracentrifugation has been examined by electron microscopy and by gel chromatography. In the first technique, freeze-drying to prevent aggregation and distortion followed by shadow-casting with platinum-iridium gave a clear impression of the particulate nature of the complex. The chromatographic experiments on Sephadex G200 confirmed the particle size and showed that the particle size distribution was not wide. The iron-dextran component is thus seen as a small particle of approximately 3 nm diameter when examined only as the FeOOH 'core' and of approximately 13 nm diameter when the attached dextran is also observed. A structural model of the particle is presented with the dextran attached by the terminal metasaccharinic acid units to the FeOOH component.

An aqueous iron-dextran complex*, which contains 5% iron and 20% dextran components, as described by Cox, King & Reynolds (1965), has been extensively used in the parenteral treatment of iron deficiency anaemia both by intramuscular and by intravenous injection. A general structure for the haematinic is now suggested mainly from examination of its physical properties, especially particle size.

Since the complex is made by neutralization of ferric chloride in the presence of an excess of the dextran component, it is reasonable to assume that colloidal ferric oxyhydroxide (FeOOH) is formed with the dextran acting as the stabilizing hydrophilic colloid attached in some way to it. The first electron microscopy studies of the complex by Muir & Golberg (1961) showed electron-dense, spherocolloidal particles 2–3 nm in diameter, which are presumed to be the colloidal FeOOH aggregates, while Ricketts, Cox & others (1965) observed similar particles 3–4 nm in diameter in the fractionated complex. However, Hall & Ricketts (1968) in referring to the earlier publication apparently misquote the particle size as being 3–4 'μ'. This together with references by Henderson & Hillman (1969) and by Hillman (1970) to a particle size of 3 μm has raised doubts on the particulate nature of the complex.

Marshall & Rutherford (1971) recently compared the physical properties of three iron-carbohydrate complexes, including the iron-dextran complex: independent evidence for the size of the particle of this complex was obtained from examination of its rate of diffusion through calibrated sinter discs.

By an application of the Stokes-Einstein equation, the calculated diameter, assuming a spherical shape, is 5·4 nm which is comparable with that of the electron-dense core observed by electron microscopy.

In the same communication, electron microscopy studies again showed the particle size of the electron-dense component in the iron-dextran complex to be approximately 3 nm in diameter. Shadow-casting revealed larger particles, with an average

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diameter of 9 nm showing that the particles consist of an electron-lucent layer, presumably of dextran, surrounding the electron-dense FeOOH nucleus. However, a tendency for the individual particles to aggregate in large masses was noted.

METHODS AND MATERIALS

Electron microscopy was carried out at the Shirley Institute, Manchester, on an AEI EM6G instrument.

For freeze-drying, the microscope grids were placed on a copper block assembly and a carbon film applied as the support for the sample. The whole assembly was then cooled with solid carbon dioxide in ethanol. The complex was sprayed onto the grids with a fine chromatographic spray before the assembly was cooled again and set in the vacuum chamber of a coating unit. A copper bar cooled with liquid nitrogen was introduced into the chamber as a cold trap after which the chamber was pumped down to 10^{-4} Torr or better in approximately 3 min.

When sublimation had been carried out for the specified period the samples were shadowed with platinum-iridium at an angle of 15° to the surface. The grids were examined at 80 KV.

The *gel chromatography* experiments were conducted on a column (60 × 1·5 cm) of Sephadex G200 (Pharmacia (G.B.) Ltd.); 0·25 ml portions of the complex or of the same solution treated to remove dextran were applied to it. 0·1M sodium acetate solution (pH 4·95) was used instead of water as the eluant to avoid staining of the Sephadex with iron. The eluate was monitored continuously for optical activity using a Bendix model 143C automatic polarimeter for the iron-dextran samples and 'Blue Dextran' (Pharmacia (G.B.) Ltd.) applied to mark the void volume. The elution of the calibrating ferritin sample (horse spleen ferritin (Koch-Light Ltd)) was monitored with a spectrophotometer operating at λ_{max} 431 nm.

Ultracentrifugation of the complex was carried out on an MSE 'Superspeed 65' ultracentrifuge at 60 000 rev/min in 3 successive runs over 20 h. The clear supernatant containing the uncomplexed dextran was removed and the iron-containing residue made up to the original volume of solution at the end of each run. The total solid content was thus reduced overall from 30 to 20% (w/v).

RESULTS AND DISCUSSION

Particle size examination

The present investigations were intended to confirm the particulate nature of the complex as a small FeOOH core surrounded by dextran and on that basis to suggest a general structure for its iron-dextran component.

The microscopy technique used was adopted to improve the resolution of the particles by minimizing aggregation. A limited sublimation time (3 h) produced impressions of single particles with others contained in gummy masses (Fig. 1A). After sublimation for 18 h, most of the particles were obscured by fibre-like material (Fig. 1B) probably due to uncomplexed dextran which previous ultrafiltration studies by Ricketts & others (1965) had shown to be present as 42% of the total dextran content. Electron photomicrographs of dextran after freeze-drying show a similar appearance (Rak, 1968). To investigate this, most of the component was removed by repeated ultracentrifugation of the complex. Electron microscopy of the residual iron-dextran after freeze-drying followed by shadow-casting of the sample gave a

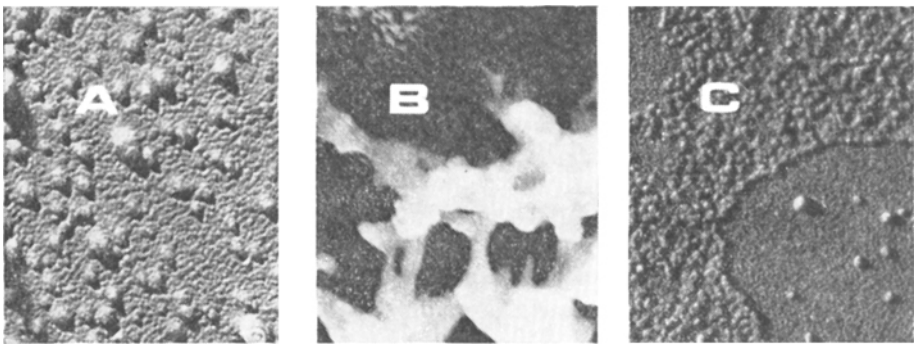

FIG. 1. A, B. Electron microscopy of the complex: A, after limited freeze-drying (3 h) and B, 18 h freeze-drying and shadow casting. A shows a gummy appearance, B shows fibrous masses ($\times$ 10^5). C. Electron microscopy of the complex ultracentrifuged to remove most of the uncomplexed dextran, after shadow casting. The particulate nature of the complex is seen ($\times$ 10^5).

much better impression of the particulate nature of the complex (Fig. 1C). The particle sizes are the largest so far observed, ranging from 11 to 21 nm with an average value of 14 nm, inclusive of the platinum-iridium layer 1 nm thick. The maximum size observed in the freeze-dried sample is consistent with the dextran remaining in an expanded state on freeze-drying from solution.

Gel chromatography on Sephadex G200 was used to compare the samples of the complex before and after ultracentrifugation and to provide additional evidence of the particle size of the iron-dextran component.

Fig. 2 shows that no changes in the iron-dextran peak occurred after ultracentri-

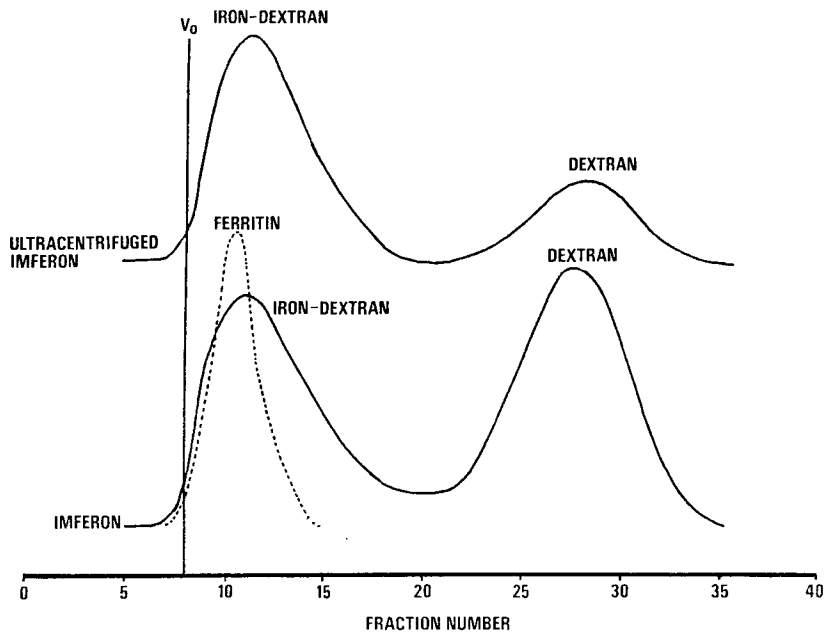

FIG. 2. Gel chromatographic profiles on Sephadex G200 of the complex before and after and ultracentrifugation showing the iron-dextran complex and unbound dextran as separate peaks. The y-axis is the response of the polarimeter (α_{Na}) for the complex samples and of a spectrophotometer (λ_{max} 431 nm) for the ferritin sample.

fugation, inferring that little or no changes had been caused in the size of the iron-dextran particles, but the excess dextran had been mostly removed.

Calibration of the gel chromatographic column with 'Blue Dextran' (Pharmacia (G.B.) Ltd.) and horse spleen ferritin (Koch-Light Ltd.) revealed that the iron-dextran complex and ferritin were eluted at almost the same elution volume. Since Determann (1969) claims that the value is mainly dependent on molecular size and as ferritin had been shown by Harrison (1963) to have a diameter of approximately 12 nm, this seemed further confirmation of the size of the iron-dextran complex.

The diameter of spherical molecules can be calculated from a graph established by Laurent & Killander (1964), relating the radius to a value K_{av}, given by $K_{av} = (V_e - V_o)/(V_t - V_o)$ (where V_t = the total volume of the gel, V_e = the elution volume for the eluted component and V_o = the void volume of the column—as determined with 'Blue Dextran'). When the calculation was applied to the iron-dextran component, a value of 12 nm for the diameter of the particle resulted (for $K_{av} = 0{\cdot}119$ corresponding to the maximum peak height).

All the sample of complex applied was eluted after the void volume, indicating that no large particles were present. Moreover, the gel chromatographic profile does not indicate that the complex has a wide size distribution, contradicting previous statements by Henderson & Hillman (1969) and by Hillman (1970) that it contains widely disparate particle sizes.

All the evidence points to a small particle size approximately 3 nm diameter when examined only as the iron 'core' and approximately 13 nm when the whole particle including the dextran layer is observed after shadow-casting. The dimensions quoted and the gel chromatography distribution curve are typical of several samples of the iron-dextran complex examined.

It is possible that the large particle sizes attributed by other workers to the complex result from their observing the large electron-dense aggregations of particles that readily occur when the material is dried on an electron microscope grid, an effect probably enhanced by the binding action of the excess dextran normally present.

General structure of the complex

The detailed physical studies of Marshall & Rutherford (1971) on the iron-dextran and two other iron-carbohydrate complexes indicate that the iron-containing cores most probably contain β-FeOOH, as shown by *X*-ray, and electron diffraction and infrared spectral evidence, although the presence of the associated carbohydrate prevented firm assignments. This is in agreement with the observation made by Müller (1967) that complex formation with carbohydrates occurred only with β-FeOOH and indeed only with a sub-class of the β-form. Gallagher & Phillips (1969) have drawn attention to the fact that the OH groups in β-FeOOH are free or only very weakly hydrogen-bonded in comparison with those in α- and γ-FeOOH. The availability of the OH groups in β-FeOOH may be the reason for its unique complexing behaviour with carbohydrates.

The alkali-treated dextran of M_n about 3500 used in the complex and the metasaccharinic acid end-units it contains have already been described by Bremner, Cox & Moss (1969). Fujita & Terato (1968) have suggested that in their studies of the absorption of dextran on ferric oxide, absorption occurred through the terminal reducing glucose unit in the dextran chain. Our experience with the complex and similar preparations shows that only when this terminal glucose is converted into a

derivative such as metasaccharinic acid does complexing occur; this suggests that the altered end unit is the means of attachment to the surface of the FeOOH component. It is therefore visualized that in solution the dextran chains radiate randomly from the FeOOH 'core' and are attached to it by the terminal metasaccharinic acid units, forming an electron-lucent sheath (Fig. 3).

Based on this model and assuming a spherical core of β-FeOOH 3 nm in diameter, a molecular weight of 73 000 has been calculated for the complex, from its chemical analysis (personal communication from R. Minshull, Fisons Limited, Pharmaceutical Division, Holmes Chapel).

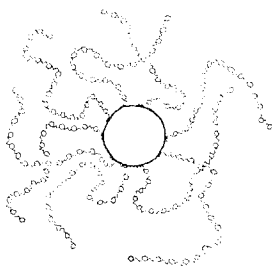

FIG. 3. Two-dimensional representation of the iron-dextran complex depicting the dextran chains attached to the FeOOH 'core'.

The complex is stable and no signs of precipitation have been noted over an extended period of several years both at ambient temperature and at 37°. These facts, together with the ability of the complex to pass through a gel-chromatography system intact, indicate that the protective dextran is firmly attached to the FeOOH component.

Acknowledgement

The part played by the Shirley Institute, Manchester, and in particular by Mr. S. C. Simmens of that Institute in carrying out the electron microscopy experiments is gratefully acknowledged.

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