

CHROM. 5296

Tritium exchange in Sephadex® G-10

Deuterium of deuterated water exchanges freely with the hydroxyl hydrogens of glucose¹, amylose and starch² even if the latter contains crystalline regions. In cellulose, however, only the hydroxyl groups in the amorphous regions are apparently accessible to deuterium^{3,4}. Since an X-ray powder diagram of Sephadex G-10 did not indicate any crystallinity⁵ it is to be expected that tritium of tritiated water will exchange completely or almost completely with the gel hydroxyls, and some exchange has been reported briefly in G-25⁶. Since, however, complete information regarding the cross-linking structure of the gel is not available it is not possible to predict with any precision the magnitude of the expected exchange. A very rough calculation of this value was made from an approximate value of the cross-linking density (kindly provided by Mr. B. SÖDERQVIST) by assuming, what is almost certainly, an oversimplified version of the cross-linking structure. The cross-links were assumed to be all single without any polymerisation into chains or branching at their non-terminal hydroxyl groups; half of the units were assumed to be linked to two dextran chains and thus to form complete cross links, while the other half were assumed to be fixed to the dextran chains at one end only.

Assuming, further, that the equilibrium constant of the exchange reaction is unity², the ratio between the number of exchange sites (hydroxyl groups) in the gel matrix (dextran chains and cross-linking structures) and the number of sites in the imbibed water of the gel (two exchange sites per water molecule) should be about 0.1. This means that the K_d value for tritium loaded as tritiated water (HTO) would be about 1.1 and provided, as seems to be the case at least under certain conditions, that column experiments are quasi-equilibrial, the column K_d value for tritium should also be the same. The exchange reaction will thus retard the movement of tritium introduced as HTO along the column. If water labelled with an oxygen isotope is eluted, the isotopic oxygen should move through the column at the same rate as water, since ¹⁸O of H₂¹⁸O does not exchange with hydroxyl or carboxyl groups⁷, of which there are also a few of the latter in the G-type Sephadex gels. Thus, the difference between the elution volumes of H₂¹⁸O and tritium of HTO should be an estimate of the extent of the tritium exchange.

Since it can be shown that the ratio between the number of hydroxyls in the gel matrix and the number of water molecules in the imbibed water of the gel increases with decreasing water regain, the G-10 gel should exhibit the greatest exchange among the members of the G-type series at present available.

Experimental

Tritiated water (Radiochemical Centre, Amersham, Great Britain) and H₂¹⁸O (loading concentration 0.4 atoms excess per cent, obtained from Nuclear Equipment Chemical Corp., New York, U.S.A. as 97 % H₂¹⁸O) were eluted together with Dextran® 500 ($\bar{M}_w = 450000$, Pharmacia Ltd., Uppsala, Sweden) as void volume indicator through a column (60 cm × 0.8 cm²) of Sephadex G-10 (Batch No. 2154). The volume of the loading solution was 0.5 ml.

Dextran was determined by an anthrone method⁸, tritium by liquid scintillation

(Beckman C PM 200) and ^{18}O of H_2^{18}O was exchanged with CO_2 (refs. 9, 10) and determined as $\text{C}^{18}\text{O}^{16}\text{O}$ in a mass spectrometer (Consolidated Electronics Corp., Model 21/201).

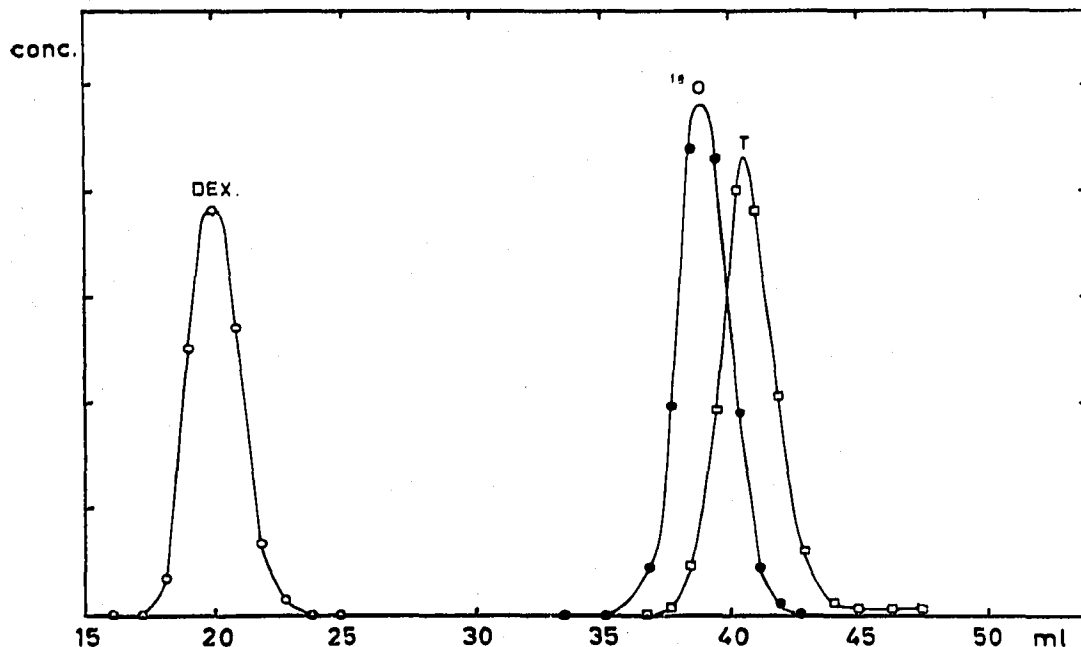


Fig. 1. Elution diagram for Dextran 500, tritium (T) and ^{18}O ; the latter two components were introduced on to the column as labelled water.

The results of an experiment are shown in Fig. 1. The ^{18}O peak precedes that of tritium and if the distribution coefficient (K_d) is defined as

$$K_d = \frac{V_T - V_0}{V_w - V_0} \quad (1)$$

where V_T , V_w and V_0 are the elution volumes of tritium, ^{18}O and dextran, respectively, the K_d value of tritium equals 1.091, which is quite near the value of 1.1 predicted above. This value means that if tritiated water is used as a reference solute a correction must be made, in calculating K_d values since tritiated water gives an overestimate of the water space in the column and hence an underestimate of the K_d value.

Thus, if⁶

$$K_{T_i} = \frac{V_i - V_0}{V_T - V_0} \quad (2)$$

and

$$K_{d_i} = \frac{V_i - V_0}{V_w - V_0} \quad (3)$$

where i refers to any solute, then

$$K_{d_i} = \phi K_{T_i} \quad (4)$$

where

$$\phi = \frac{V_T - V_0}{V_w - V_0}$$

For G-10, ϕ is thus 1.09, while for G-25 it is about 1.06 (ref. 6). ϕ will become increasingly smaller as the water regain value increases and should be less than about 1.02 in G-200.

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