Acta Chemica Scandinavica B 28 (1974) 777—780

Radiation Induced Effects on Exchangeable Hydrogens in Crystalline Carbohydrates

GÖRAN LÖFROTH and TORBJÖRN GEJVALL

Radiobiology Department, University of Stockholm, Wallenberg Laboratory, Lilla Frescati, S-104 05 Stockholm 50, Sweden

Crystalline mono- and disaccharides, unlabeled and labeled with deuterium or tritium in the exchangeable positions, were irradiated with ^{60}Co γ-rays. Hydrogen gas, H_2, is formed with G-values of 3.0 – 3.8 in unlabeled carbohydrates. Mass spectrometry of the hydrogen gas formed in the deuterium labeled carbohydrates showed that only 2 – 4 % is in the form of HD and that no D_2 is detectable. Irradiation of the tritium labeled carbohydrates transfers tritium to non-exchangeable positions and a large part of this tritium is present in the original carbohydrate. G-Values of 1 – 2 can be calculated for the transfer process assuming that no isotope effect is present. The results indicate that the radiation induced transfer from exchangeable positions to non-exchangeable positions in crystalline carbohydrates occurs at an early stage following the absorption of the radiation.

Radiation induced hydrogen transfer from exchangeable sites to non-exchangeable sites has been detected in several types of compounds in the solid state.[1,2] In amino acids, labeled with deuterium in the exchangeable positions, transfer of exchangeable hydrogen to carbon-bound hydrogen in the radiation induced free radicals has been investigated by ESR (Ref. 1 and quoted refs.). Smitherman *et al.*[2] have studied transfer from exchangeable to non-exchangeable sites in lyophilized samples of proteins, nucleic acid and D-glucose by means of tritiated compounds and have suggested that destruction of secondary radicals may be responsible for the radiation induced hydrogen transfer.

In the present investigation the extent of the radiation induced hydrogen transfer to positions at carbon atoms has been determined in some crystalline carbohydrates labeled with tritium in the exchangeable positions. In parallel studies with non-labeled samples and samples labeled with deuterium in the exchangeable positions quantitative determinations of the radiation induced formation of hydrogen gas, H_2, HD, and D_2, have been performed in order to evaluate the involvement of the exchangeable hydrogens in the formation of hydrogen gas.

EXPERIMENTAL

The ^{60}Co γ-irradiation source and the non-labeled carbohydrates have previously been described.[3]

Labeling of the exchangeable hydrogens in the carbohydrates was made by crystallization in aqueous solvents containing tritiated or deuterated water. The specific activities of the tritiated samples were in the range 22 – 76 mCi/mol. The theoretical exchange of deuterium for hydrogen, as calculated from the ratio of the number of exchangeable deuterium atoms to the number of exchangeable hydrogen plus deuterium atoms in the crystallization solution, ranged from 55 % (in sucrose) to 87 % (in α-D-glucose).

The tritiated carbohydrates were irradiated in the presence of air and then dissolved in water bubbled with nitrogen. The water was distilled off at reduced pressure and temperatures not exceeding 35 °C. Dissolution of the residue in water and distillation were repeated until all exchangeable tritium had been removed and the solid residue had a constant specific activity. This took about eight cycles. Syntheses of di-*O*-isopropylidene-D-fructose and di-*O*-isopropylidene-D-glucose were made directly on the unirradiated and irradiated samples whereas syntheses of the octa-*O*-acetyl derivatives of sucrose and trehalose were made on

samples from which the exchangeable tritium had been removed. Di-*O*-isopropylidene-D-glucose was prepared according to Glen *et al.*[4] and the other derivatives were synthesized and purified as described earlier.[3] The determination of radioactivity was mainly done with aqueous scintillation with Instagel (Packard Inc.).

For the determination of hydrogen gas the samples were irradiated in sealed glass ampoules. After irradiation the ampoule was opened when attached to a small flask having a second side arm closed with a rubber membrane through which water was added for dissolution of the sample and head space gas withdrawn for analysis. Hydrogen gas was determined by gas chromatography with a 2.5 m × 2.1 mm Molecular Sieve 5A 60 – 80 mesh column at 50 °C and with hot wire detection at 100 °C. The determination of HD and D_2 relative to H_2 was made on a CH-4 Atlas M-A-T mass spectrometer at the Mass Spectrometry Laboratory, Karolinska Institute, Stockholm, on the head space gas of samples irradiated *in vacuo* and on the head space gas of samples irradiated in the presence of air and then dissolved in water *in vacuo*.

RESULTS

Labeling of the carbohydrates with tritium in the exchangeable positions occurred to the extent expected from the amount of added tritium and the number of exchangeable positions in the carbohydrates and the crystallization solvent. This indicates that no measureable isotope effect is present in the recrystallization labeling procedure.

Upon irradiation, followed by removal of the exchangeable tritium by repeated distillation, a dose related increase of non-exchangeable tritium was present in the samples (*cf.* Figs. 1 and 2). Except for trehalose dihydrate the tritium incorporation is linearly dependent on the dose up to at least about 10^{21} eV/g. With the assumption that no isotope effect is operating in the transfer process the following

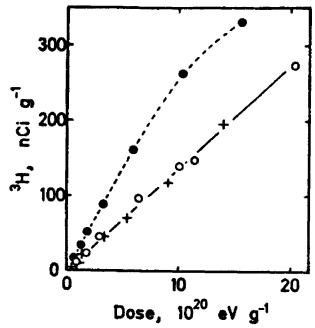

Fig. 1. The radiation induced transfer of ^3H from exchangeable to non-exchangeable positions in crystalline carbohydrates labeled with ^3H in the exchangeable positions (mCi/mol carbohydrate); dose *vs.* amount of non-exchangeable ^3H per g irradiated carbohydrate; ● D-fructose 35 mCi/mol, O α-D-glucose 22 mCi/mol, and + α-lactose.H_2O 66 mCi/mol.

Table 1. Radiation induced transfer of tritium in crystalline carbohydrates from exchangeable positions to non-exchangeable positions determined after (a) repeated evaporation of water and (b) derivatization and purification with respect to the original carbohydrate.

Compound and sp. activity	Dose 10^{20} eV/g	nCi non-exchangeable ^3H per g[a] (a) of irradiated sample	(b) of original carbohydrate
D-Fructose 35 mCi/mol	5.9	160	110
	10.4	264	165
	16.0	331	249
α-D-Glucose 22 mCi/mol	10.1	139	108
Sucrose 61 mCi/mol	11.4	113	63
Trehalose·$2H_2O$ 76 mCi/mol	15.8	214	72

[a] After correction for tritium incorporation in non-exchangeable positions in unirradiated samples.

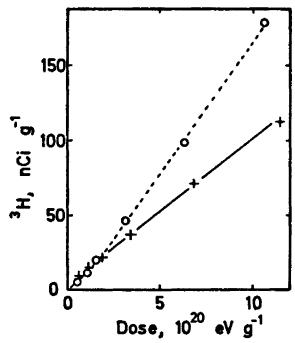

Fig. 2. As for Fig. 1 with O trehalose.$2H_2O$ 76 mCi/mol and + sucrose 61 mCi/mol.

G-values are obtained: D-Fructose 2.3, α-D-glucose 1.8, α-lactose monohydrate 1.2, and sucrose 0.8. In the case of trehalose dihydrate there is an exponentially increasing dose response up to about 10^{21} eV/g (Fig. 2) with an initial G-value of about 0.9 and a slope in the dose region $0.5 \times 10^{21} - 10^{21}$ eV/g corresponding to a G-value of about 1.7. A G-value of 0.95 for transfer of exchangeable hydrogens to non-exchangeable positions has been reported for lyophilized D-glucose samples, the modification of which was not reported.[2] Tritium is also present in non-exchangeable positions in unirradiated samples; these incorporations were generally small (*i.e.* 0.3 – 5 nCi/g) compared with the radiation induced incorporations and they have been corrected for in the calculations.

By preparation of the di-*O*-isopropylidene derivative directly from unirradiated and irradiated D-fructose it was found that most of the radiation induced tritium incorporation (about 70 %) is present in D-fructose itself (Table 1). A similar investigation on the other carbohydrates by means of the suitable acetyl derivatives resulted in a high tritium incorporation into the acetoxy methyl group. Derivatization after removal of the exchangeable tritium was, however, feasible and analyses of sucrose and trehalose dihydrate showed that a large part of the radiation induced incorporation is in the original carbohydrate (Table 1). The same seems to hold also for α-D-glucose as determined by analyses of its di-*O*-isopropylidene derivative prepared directly from the crystalline samples; in this case, in contrast to D-fructose, an appreciable tritium incorporation (178 nCi/g) occurred in the unirradiated sample. The difference may be that the D-fructose derivative is prepared with only anhydrous acetone and zinc chloride whereas also the presence of another acid, *e.g.* phosphoric acid, is necessary for D-glucose.

Analyses of hydrogen gas formation in the dose range $10^{19} - 4 \times 10^{20}$ eV/g showed that the total yield, obtained after dissolution of the samples, is linearly dependent on the dose; $G(H_2)$ was found to be 3.8 for α-D-glucose, lactose monohydrate, and maltose monohydrate 3.1 for sucrose and 3.0 for D-fructose and trehalose dihydrate. Depending on the type of carbohydrate, various portions of the hydrogen gas are released from the crystals in the solid state; the per cent released was 15 – 25 for α-D-glucose, D-fructose and sucrose, 50 – 60 for lactose monohydrate, and 70 – 80 for maltose monohydrate and trehalose dihydrate. The present result on hydrogen gas formation in α-D-glucose is the same as that reported by Phillips and Baugh.[5]

Mass spectrometric analyses of the relative amount of HD to H_2 performed on α-D-glucose, D-fructose, sucrose, and trehalose dihydrate deuterated in the exchangeable positions and irradiated with doses of about 1.5×10^{20} eV/g showed that only 2 – 4 % of the hydrogen gas was in the form of HD. Analyses of D_2 was usually not possible, probably due to contamination from helium, except for one analysis of each of α-D-glucose, sucrose, and trehalose dihydrate; in these analyses no peak was detected at mass number 4 while the HD was detected; this result indicates that D_2 at most is only one fifth to one tenth of HD. Control experiments with non-labeled samples dissolved in D_2O after irradiation showed that no or negligible amounts of HD were formed.

DISCUSSION

Several mechanisms have been discussed for the radiation induced hydrogen transfer from exchangeable to non-exchangeable sites.[2] Among these are reactions involving hydrogen atoms and molecular hydrogen. The present data show that no more than a very small part of the radiation induced hydrogen gas is derived from the exchangeable hydrogens in the carbohydrates studied. This infers that neither

hydrogen gas nor such hydrogen atoms which are precursors to hydrogen gas play a significant role in the radiation induced hydrogen transfer from exchangeable to non-exchangeable positions.

Exchange reactions of secondary radicals, as observed in amino acids,[1] are not likely mechanisms, as irradiated polycrystalline carbohydrates,[6] and single crystals of sucrose [7] and trehalose [8] deuterated in exchangeable positions show the same ESR spectra as non-labeled samples.

The radiation induced transfer of hydrogen to non-exchangeable sites, as measured by tritium incorporation, is, except for trehalose dihydrate, linearly dependent on the dose over a very large dose range. In the same dose range, formation of stable, secondary free radicals occurs with decreasing efficiency with increasing doses; this also occurs at doses below 2×10^{19} eV/g for sucrose.[9] It is thus unlikely that removal of secondary radicals is related to the tritium incorporation. The fact that a large part of the tritium incorporated into non-exchangeable positions is present in the original carbohydrate favours an interpretation that the transfer occurs at an early stage following the absorption of the radiation.

Further studies, particularly to investigate into which position(s) the exchangeable hydrogens are incorporated, may give additional insight into the transfer mechanism and may also lead to methods to synthesize labeled carbohydrates.

Acknowledgement. This investigation has been supported by grants from the Swedish Atomic Research Council.

REFERENCES

1. Grinberg, O. Ya., Serdobov, M. V. and Lebedev, Ya. S. *Biofizika 17* (1972) 208.
2. Smitherman, T. C., Moss, H. M. and Riesz, P. *Radiation Res. 46* (1971) 251.
3. Löfroth, G. *Int. J. Radiat. Phys. Chem. 4* (1972) 277.
4. Glen, W. L., Myers, G. S. and Grant, G. A. *J. Chem. Soc.* (1951) 2568.
5. Phillips, G. O. and Baugh, P. *J. Chem. Soc. A* (1966) 370.
6. Löfroth, G. *Unpublished data.*
7. Gräslund, A. *Personal communication.*
8. Gräslund, A., Löfroth, G. and Edshammar, L.-E. *Book of Abstracts, IVth Intern Congr. Radiation Res.*, Evian 1970, Abstract No. 321.
9. Löfroth, G., Ehrenberg, L. and Ehrenberg, A. *Fifth Intern. Symp. Free Radicals*, Almqvist & Wiksell, Stockholm 1961, Paper No. 38.

Received March 8, 1974.