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DETERMINATION OF CHEMICALLY BOUND OXYGEN BY ^{15}O-O TRACER METHOD. APPLICATION TO HEMOGLOBIN- AND SYNTHETIC HEME-BOUND OXYGEN

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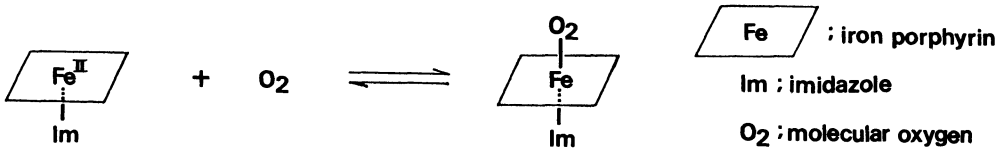
Oxygen-15 radiotracer method was applied to the determination of chemically bound oxygen. Volume of oxygen bound with red blood cell suspension or with synthetic heme was evaluated within an error of ca. ±5%.

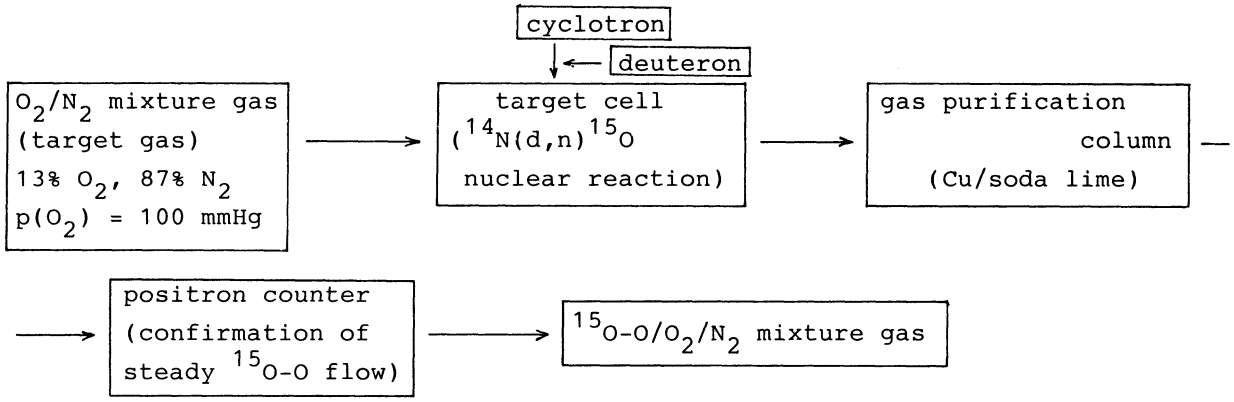
Oxygen dissolved in water is commonly measured by oxygen probe technique.[1] Oxygen dissolved in a solution can be determined quantitatively by this method, though it is sensitive only to molecular oxygen. Chemically bound oxygen has been determined by volumetric method, Warburg method,[2] van Slyke method[3] and so on up to the time. However, these methods have to be carefully operated by skilled workers using a closed vessel under a constant temperature for a long time to suppress the effect of chemical reaction heat. Thus, possible error in these measurements often can not be disregarded.

Oxygen-15 is a cyclotron-produced positron nuclide formed by the nuclear reaction $^{14}N(d,n)^{15}O$ and the subsequent spontaneous isotope exchange reaction, $^{15}O + O_2 \longrightarrow {}^{15}O\text{-}O + O$.[4] This nuclide decays with a half life ca. 120 s and can be detected efficiently by the measurement of the positron annihilation radiation. The ^{15}O-O tracer method has been used in vivo for the study and diagnosis of functions of lungs in a living body.[5]

In this paper, we intend to show the ^{15}O-O tracer method applied to the determination of chemically bound oxygen volume using the example of hemoglobin- and synthetic heme-bound oxygen. Molecular oxygen is absorbed reversibly by hemoglobin in blood and a synthetic heme by the coordination bond (Scheme 1). This reaction plays important roles in various in vivo and in vitro chemical changes. The samples were a red blood cell suspension for hemoglobin[6] and a liposomal



Scheme 1.



Scheme 2. Supply line of the ^{15}O-O tracer.

heme for the synthetic heme. The latter is an artificial oxygen carrier synthesized by us[7,8]: Synthetic hemes are embedded in the bilayer of lipid liposome with radius of 400 Å.

The line of supply for the ^{15}O-O tracer was shown in Scheme 2. Gas mixture of O_2 and N_2 ($p(O_2)$ = 100 mmHg) was conducted to the target cell and bombarded with deuterons accelerated by a cyclotron. Atomic ^{15}O was formed via the $^{14}N(d,n)^{15}O$ nuclear reaction and the oxygen-15 molecule was given by the spontaneous isotope exchange reaction. The crude ^{15}O-O/O_2/N_2 mixture gas was passed through a Cu/soda lime column (φ 10 mm x 100 mm) to remove NO_x and ^{17}F formed by the radiation effect and by the $^{16}O(d,n)^{17}F$ reaction, respectively. Thus, ^{15}O-O/O_2/N_2 ($p(O_2)$ = 100 mmHg) mixture gas was obtained. The mixture gas was supplied with a steady flow (200 ml/min) and a steady radioactivity intensity (150 μcpm).

Before the determination of oxygen absorbed by the samples, measurements were made to certain (i) the radiochemical purity of the supplied gas, (ii) the attainment of the absorption saturation, and (iii) the absence of the ^{15}O isotope exchange between ^{15}O-O and H_2O. By sampling the mixture gas, the half life of the radioactivity was determined to be ca. 124 s, which almost agreed with that in the reference 4 (120 s). The chemical species of radioactive gas is thus confirmed to be ^{15}O-O. The ^{15}O-O mixture gas was bubbled through sample solutions, i.e. the red blood cell suspension and the liposomal heme solution at a constant temperature, and the annihilation radiation intensity of the solution was measured by sampling at 30 s intervals. The intensity became saturated after a few minutes. The saturation of oxygen-binding was also confirmed by the observation that visible absorption spectrum change from the deoxy heme to the oxygen adduct reached to a saturation point after a few minutes by bubbling of the same pressure oxygen gas through the sample solutions. After the ^{15}O-O mixture gas had been bubbled through the sample, the sample was ultra-centrifuged to separate the red blood cell or the liposomal heme. The colorless supernatant without the liposomal heme was isolated and its annihilation radiation intensity was measured. Above operation was carried out within ten minutes. The intensity of the supernatant

Table 1. Oxygen uptake by red blood cell and liposomal heme

Sample (Heme concn.)	Annihilation radiation intensity (t=0, cpm/ml)	Standard deviation %	Oxygen uptake volume (ml O_2/100 ml) Present method	O_2-probe	Spectroscopy	Relative % (Present method /Spectroscopy) x 100
Red blood cell						
(0.5 mM)	2970	4.0	1.57	0.415	1.64	96
(1.0 mM)	5560	4.8	2.94	0.417	2.86	103
Liposomal heme						
(1.0 mM)	3540	4.2	1.87	0.423	2.04	92
Aqueous media[a)]	795	1.8	0.421	--	--	--
^{15}O-O gas	24900	-	--	--	--	--

At 30°C, 760 mmHg and p(O_2) = 100 mmHg. [a)]Aqueous media; distilled water, phosphate buffer solution, and phospholipid liposome solution.

was consistent with that of the water through which the ^{15}O-O mixture gas was bubbled under the same condition. This means that ^{15}O-O isotope exchange reaction with H_2O (^{15}O-O + H_2O ⟶ O_2 + $H_2{}^{15}O$) did not occur.

Oxygen uptake by the samples was measured from the ^{15}O intensity of them. Each value was obtained as the average over ten samples, within the error of ±5%. The oxygen uptake by the red blood cell suspension and the liposomal heme (in ml O_2/100 ml sample) were calculated from the annihilation radiation intensity of the aqueous media under the same conditions and reported oxygen solubilities in the aqueous media.[9)] Annihilation radiation intensity of the aqueous media agreed with one another within experimental error. Oxygen solubility in water is known to be 26.10 ml O_2/l at 30°C. The heme-bound oxygen was also determined by the absorbance of the deoxy heme and by oxygen-binding equilibrium measurement; the oxygen-binding percentage were 98% and 65% for the suspension of the red blood cell and the liposomal heme, respectively. These results are all shown in Table 1.

The volumes of oxygen absorbed by the red blood cell suspensions agreed with those calculated from spectrophotometric determination; this supports the validity of the measurement. The volume of oxygen absorbed by the liposomal heme solution was found to be 1.87 ([heme] = 1.0 mM) ml O_2/100 ml solution, which is close to the calculated value (2.04 ml O_2/100 ml solution). For reference, the values determined by O_2 probe method were also shown in Table 1. By this method, volumes of absorbed oxygen were found to be all ca. 0.42 ml O_2/100 ml solution for the red blood cell suspension, the liposomal heme solution and the aqueous media. This indicates that the O_2 probe method gives physically dissolved oxygen volume. That is, the ^{15}O-O tracer method can evaluate overall absorbed oxygen volume involving chemically bound oxygen volume. Further study of quantitative oxygen measurement

in a living body will be reported in future.

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