Modelling $^{18}O_2$ and $^{16}O_2$ unidirectional fluxes in plants: I. Regulation of pre-industrial atmosphere

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A B S T R A C T

In closed systems, the O_2 compensation point (Γ_O) was previously defined as the upper limit of O_2 level, at a given CO_2 level, above which plants cannot have positive carbon balance and survive. Studies with $^{18}O_2$ measure the actual O_2 uptake by photorespiration due to the dual function of Rubisco, the enzyme that fixes CO_2 and takes O_2 as an alternative substrate. One-step modelling of CO_2 and O_2 uptakes allows calculating a plant specificity factor (Sp) as the sum of the biochemical specificity of Rubisco and a biophysical specificity, function of the resistance to CO_2 transfer from the atmosphere to Rubisco. The crossing points (Cx, Ox) are defined as CO_2 and O_2 concentrations for which O_2 and CO_2 uptakes are equal. It is observed that: (1) under the preindustrial atmosphere, photorespiration of C3 plants uses as much photochemical energy as photosynthesis, i.e. the Cx and Ox are equal or near the CO_2 and O_2 concentrations of that epoch; (2) contrarily to Γ_C, a Γ_O does not practically limit the plant growth, i.e. the plant net CO_2 balance is positive up to very high O_2 levels; (3) however, in a closed biosystem, Γ_O exists; it is not the limit of plant growth, but the equilibrium point between photosynthesis and the opposite respiratory processes; (4) a reciprocal relationship exists between Γ_O and Γ_C, as unique functions of the respective CO_2 and O_2 concentrations and of Sp, this invalidates some results showing two different functions for Γ_O and Γ_C, and, consequently, the associated analyses related to greenhouse effects in the past; (5) the pre-industrial atmosphere levels of O_2 and CO_2 are the Γ_O and Γ_C of the global bio-system. They are equal to or near the values of Cx and Ox of C3 plants, the majority of land plants in preindustrial period. We assume that the crossing points represent favourable feedback conditions for the biosphere-atmosphere equilibrium and could result from co-evolution of plants–atmosphere–climate. We suggest that the evolution of Rubisco and associated pathways is directed by an optimisation between photosynthesis and photorespiration.

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1. Introduction

The role of land plants in the equilibrium of oxygen (O_2) and carbon dioxide (CO_2) concentrations in the atmosphere was reviewed by Igamberdiev and Lea (2006), with analysis of numerous results

of geochemical models, able to predict variations in palaeoatmospheric composition from geological samples and isotopic data.

Two epochs are particularly fascinating: (i) Late Carboniferous, ca. 300 million years ago, for which geochemical models, using palaeo carbon and sulphur cycles, calculate a possible level of O_2 up to 35% and CO_2 of 0.03% (300 ppm). Palaeo fires served as a strong negative feedback against excessive O_2 variations (Berner, 1999; Berner and Kothavala, 2001; Lenton, 2001). In numerous studies on Phanerozoic atmosphere, the role of photorespiration was rarely evocated, with the exception of Beerling et al. (1998). More recent works (Lenton, 2001; Igamberdiev and Lea, 2006) are based on the experiments of Tolbert et al. (1995) and Beerling et al. (1998). (ii) Despite of cyclic variations (glacial phases), the pre-industrial O_2 and CO_2 concentrations tended towards relatively stable values of 21% and 280 ppm, respectively, before significant anthropogenic actions (Berner and Kothavala, 2001, and Figs. 1 and 2 in Igamberdiev and Lea, 2006). The first application of a physiological concept to the regulation of global cycles of carbon and oxygen was developed by Berry et al. (1994). The main attention was made

to the role of Γ_C on the atmosphere equilibrium. Igamberdiev and Lea (2006) concluded that photorespiration (the rate of which is directly related to the O_2/CO_2 ratio, due to the dual function of ribulose 1,5-bisphosphate carboxylase/oxygenase, i.e. Rubisco) may be an important mechanism in maintaining the limits of O_2 and CO_2 concentrations by restricting land productivity and weathering. That conclusion was particularly based on the study of Tolbert et al. (1995). The challenge of Tolbert et al. (1995) seems judiciously chosen: '*in a given plant, at a fixed CO_2 concentration, what is the O_2 concentration for which the net CO_2 assimilation of plants tends to zero*?' From the concept of compensation points of CO_2, applied to O_2 variations, they observed several O_2 compensation points as a function of CO_2 levels. Tobacco and spinach plants were studied in a closed growth chamber for several hours in different atmospheres: at 350 ppm CO_2, the net CO_2 uptake fell to zero at 27% O_2 (i.e. the O_2 compensation point was 27%); at 220 and 700 ppm CO_2, the O_2 compensation points were 23 and 35% O_2, respectively. The conclusion was made that the O_2 compensation points represent the upper limit of atmospheric O_2 (at a given CO_2 level) above which C3 plants cannot survive. Tolbert et al. (1995) suggested that such O_2 compensation points should control the rise of atmospheric O_2 level at each atmospheric CO_2 concentration.

Beerling et al. (1998) studied the effect of elevated O_2 on the acclimation of small plants growing (after germination) for 6 weeks in growth chambers at 35% O_2 and 300 ppm CO_2. Photosynthesis of leaves was measured in a cuvette chamber at different CO_2 and O_2 concentrations. Its rate was halved under 35% O_2 as expected by a biochemical model (Farquhar et al., 1980). No significant changes in acclimation were observed based on photosynthesis rates in comparison with plants maintained in a normal atmosphere of 21% O_2 and 300–350 ppm CO_2.

Without considering the possible role of compensation points observed by Tolbert et al. (1995), the biochemical model is embedded in global models that analyse Carboniferous palaeoatmospheres, mainly to introduce the action of CO_2 in photosynthesis, into the carbon balance (Berner and Kothavala, 2001; Beerling and Berner, 2005). The inhibitory effect of O_2 is introduced, but other specific roles of photorespiration, are ignored. The last paper analyses, in particular, positive feedbacks of CO_2 concentration on weathering, via its action on plant growth. The coevolution of plants and atmospheric CO_2 is noted with careful analysis of size of leaves or plant height. Igamberdiev and Lea (2006) reviewed the global results concerning respective changes in O_2 and CO_2 concentrations and, following the ideas of Tolbert et al. (1995), concluded that an important role of photorespiration was to equilibrate atmospheric O_2 and CO_2 concentrations. The finding that Rubisco is the chief architect of the atmosphere is also a conclusion of Nisbet et al. (2007) and Nisbet and Nisbet (2008). Based on the Tolbert et al. (1995) data, they found two "permitted zones" limited by the two linear functions of Γ_O and Γ_C. The borders of these zones represent the limits of two permissible stable states of photosynthesis in the domain defined by the O_2 and CO_2 concentrations. These borders limited the instabilities of climate and the greenhouse effects imposed either by methane or by CO_2 starting from about 2.3 Ga ago.

In accordance with the views of Igamberdiev and Lea (2006), the approach taken by Tolbert et al. (1995) is valid as long as it is remembered that the experiments were carried out in closed chambers over short periods of time and therefore have some limitations when applied to the whole biosphere. The enclosed system described by Tolbert et al. (1995) lacked an ocean and rock cycle, both of which regulated the atmospheric CO_2 during the evolution of land plants. In O_2 evolution, the role of photosynthesis (both marine and land) was clearly predominant, but the role of different factors in CO_2 depletion/evolution, requires numerous estimates that appear sometimes controversial.

All this mean that a simple photosynthetic/photorespiratory O_2/CO_2 balance mechanism is not sufficient to explain gaseous homeostasis of the atmosphere. However, it is admitted that the biospheric equilibrium of CO_2 and O_2 concentrations works with a feedback mechanism that may be responsible for the oscillation regime probably together with the cycles of solar activity. This indicates that the approach of Tolbert et al. (1995) has a rational basis. It was further developed by Nisbet et al. (2007) and Nisbet and Nisbet (2008) who suggested the concept of permitted zone based on the variations of compensation points as function of O_2 and CO_2 concentrations. As the data of Tolbert are not in agreement with the results of Beerling et al. (1998), the role of both CO_2 and O_2 compensation points requires further development and clarification. Not far from the Γ_C concept, the notion of ecological CO_2 threshold has been proposed and modelled by Pagani et al. (2009). That modelling combines the positive effect of plant roots in rock weathering and a negative feedback when photosynthesis decreases the CO_2 level. The CO_2 decrease is limited by an ecological threshold. A specific role of *PR* in the rock weathering was not mentioned.

The present work uses modelling of steady-state exchange data (André and Massimino, 1984) from several experiments in closed chambers with different atmospheres. The main interest of these data is the use of $^{18}O_2$ labelling to directly measure the O_2 uptake due to oxygenation, including photorespiration, its main part. The simple model allows a reexamination of the relationship between plants, atmosphere and biosystem, to clarify the role of the O_2 compensation point and its liaison with Γ_C. The second advantage is the comparison between ancient and modern plants. Using the results of modelling of *in vivo* experiments, the role of oxygenation processes in the equilibrium concentrations in the pre-industrial atmosphere is analysed. New arguments for a plant–atmosphere coevolution are proposed, in relation to Rubisco evolution.

2. Methods

2.1. Experiments with Whole Plants

The modelling of steady-state exchange data (André and Massimino, 1984) from several experiments in closed chambers with different atmospheres was performed. In contrast to many other studies of photosynthesis, the exchanges were measured in aerial parts of whole plants, the roots being isolated and measured separately (André et al., 1985). Exceptions are made for mosses and macroalgae. The major point of the measurements in closed growth chambers in an artificial atmosphere is that the whole plants were acclimated and studied in conditions near the seasonal average of field or natural parameters, as demonstrated for maize and wheat plants (André et al., 1978; Gerbaud and André, 1979, 1980). The Data were produced from steady-state daily exchanges of aerial parts. Days when treatments were applied were compared to the days under standard conditions. To compensate for the variation due to growth, quasi steady-state daily data were normalised by the daily exchanges of days under standard conditions, assuming 'standard' net CO_2 and O_2 exchanges had a reference value of one (Gerbaud and André, 1979).

2.2. $^{18}O_2$ Labelling

The CO_2 and O_2 exchanges during photosynthesis, using $^{18}O_2$ labelling, were analysed in detail by von Caemmerer (2000). In agreement with Gerbaud and André (1979, 1980) and Canvin et al. (1980), the major assumptions are:

(a) O_2 evolution is derived from water splitting at photosystem II, and there is no discrimination and thus the isotopic composition is that of the water from which it is derived. The O_2 evolution measures the electron flow generated by light in chloroplasts, and reflects the total photo-energy available for biochemical 'dark' reactions.

(b) O_2 uptake processes consume O_2 in proportion to the isotopic abundance of O_2 in the surrounding air. Thus, it has been possible, with labelled $^{18}O_2$ and mass spectroscopy, to distinguish between O_2 evolution ($^{16}O_2$) and O_2 uptake processes. O_2 uptake measures mainly the oxygenation of Rubisco, but also the Mehler reaction (Fig. 1).

(c) There is little difference between the net CO_2 and O_2 exchanges (P_C and P_O in Fig. 1), the classical measure of photosynthesis. In results and modelling, they are both expressed as P. The gas exchange of green parts of plants during light and dark periods is shown in Fig. 1. The labelling of O_2 with the stable isotope $^{18}O_2$ enables monitoring of the atmosphere in a closed chamber (Gerbaud and