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Effect of temperature and oxygen on the activity of microsomal oleyl-coenzyme A desaturase of potato tubers

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Summary

To check if the oxygen concentration in the cell medium - higher at low temperatures - is a factor regulating the synthesis of polyunsaturated fatty acids, the effects of temperature and oxygen concentration were studied in vitro on the activity of the microsomal oleyl-coenzyme A desaturase of potato tubers. The highest activity of this enzyme was observed at 30 ° C, and not at low temperatures, and at an oxygen concentration of about 20 %. These results suggest that the above hypothesis is not valid for the potato tuber cell.

Introduction

Linoleic acid is an essential fatty acid, needed for the growth of Vertebrates since the cells of these animals cannot synthesize this particular fatty acid. In higher plants, linoleic acid is formed by desaturation of oleyl-coenzyme A. This desaturation is catalysed by an oleyl-coenzyme A desaturase, associated with the microsomal pellet in cell-free systems (Vijay & Stumpf, 1971; Abdelkader et al., 1973). The activity of this enzyme may easily be studied in microsomal fractions isolated from 'aged' slices of potato tuber; during this 'ageing' process, there is a marked increase in the desaturase activity of the potato cells (Cherif, 1973).

We have begun to study systematically the properties of the oleyl-coenzyme A desaturase. One of the first questions concerned the effects of temperature and oxygen on the linoleic acid synthesis. It has been known for many years (Hilditch & Williams, 1964; Canvin, 1965) that when plants are grown at low temperatures, their polyunsaturated fatty acid content increases. To try to explain this phenomenon, Harris & James (1969) suggested that a low temperature increased the solubility of oxygen in the cellular medium; since molecular oxygen is necessary for the desaturations, these authors proposed that the temperature effect is an indirect one: the oxygen concentration may be the true regulating factor in the activity of plant desaturase since this concentration is higher at low temperature.

All the experiments of Harris & James were performed in intact tissues; it was of

interest to study in vitro the effects of temperature and oxygen concentration on the activity of the microsomal oleyl-coenzyme A desaturase of potato tuber and thus, to check if the interesting hypothesis of these authors can be applied to an in vitro system.

Methods

Thin disks (1 × 13 mm) excised from potato tuber parenchyma (cv. Bintje) were thoroughly rinsed with distilled water and were shaken in a small volume of calcium sulphate solution (10^{-4} *M*) at 25 °C. After 16 h of this treatment, the disks were ground in an ice-cold mortar with 1 volume per 1 g of the following solution: sucrose (0.4 *M*), magnesium chloride (0.01 *M*), bovine serum albumin (1 %), cysteine hydrochloride (0.004 *M*) and ethylene-diamine tetraacetate (EDTA) (0.001 *M*) in a Tris-HCI buffer (0.1 *M*) at pH 7.5. After filtration through 'Miracloth', the homogenate was centrifuged at 10 000 g for 15 min; the supernatant was centrifuged again at 100 000 g for 60 min to collect a microsomal pellet, which contained the oleyl-coenzyme A desaturase.

To follow the activity of this desaturase, an aliquot of the microsomal pellet (1 mg of protein) was introduced in an assay tube which contained, in a final volume of 3 ml, 1 μmol of reduced nicotinamide adenine dinucleotide (NADH), 10 mg of bovine serum albumin, 2 mg of magnesium chloride, 2.6 μmol of (1^{-14} C)-oleyl-coenzyme A (prepared in the laboratory (Abdelkader et al., 1973)) and 0.1 *M* sodium phospate, pH 7.0. The assay tube was vigorously shaken at a fixed temperature, for 5 to 120 min, under different gaseous mixtures.

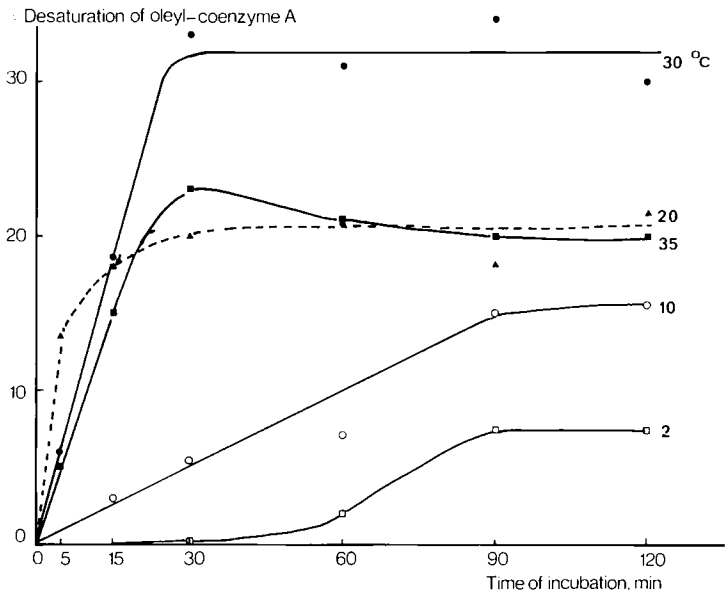
At the end of the incubation, 5 ml of methanolic sodium hydroxide (0.5 *N*) were added in the tube; the stoppered tube was kept at 70 °C for 15 min. The fatty acid methyl esters were extracted by adding 10 ml of pentane in the cooled tube. The methyl esters were then analyzed by radio GLC (Packard, model 894) under the following experimental conditions: column (3 m) of 20 % butane-diol-succinate on hexamethyldisilanized Chromosorb W at 210 °C; temperature of the combustion oven 750 °C. This technique gives simultaneously the distribution of the radioactivity between the different fatty acids and the mass composition of the fatty acid mixture. The percentage of desaturation of oleic acid was calculated from relative areas of radioactivity peaks for methyl oleate and methyl linoleate.

Results

Effect of the incubation temperature

Fig. 1 presents the kinetics of desaturation of oleyl-coenzyme A in vitro at various temperatures. It is clear that at 30 °C the highest activity of oleyl-coenzyme A desaturase (34 % of desaturation) was obtained; lower (20 °C) or higher (35 °C) temperatures decrease the intensity of desaturation. It is interesting to notice that for the first 15 min of incubation, the slope of the curve is nearly similar for these

Fig. 1 Temperature effect on the activity of the microsomal oleyl-coenzyme A desaturase of potato tuber. The incubation temperature, varying from 2 to 35 °C, is indicated in the graph.



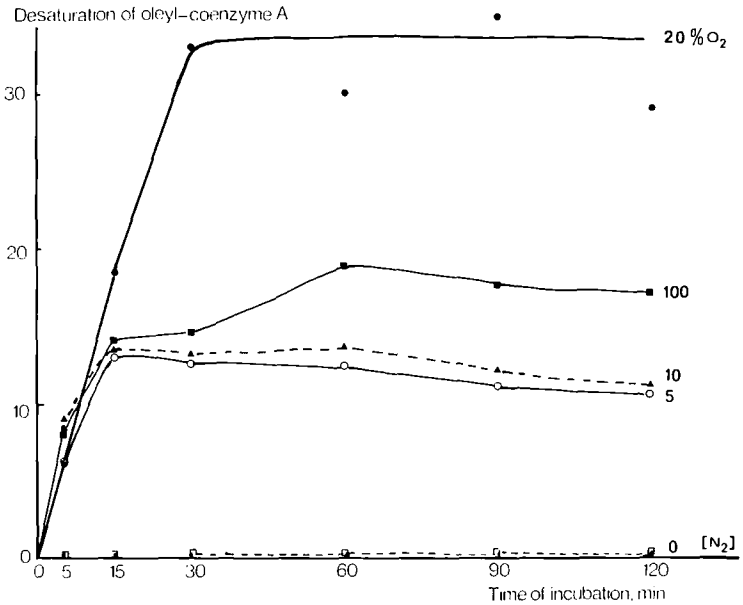
Time of incubation, min - *Inkubationszeit, in Minuten - Temps d'incubation (minutes)*
Desaturation of . . . - *Versättigung von . . . - Désaturation d' . . .*

Abb. 1. Einfluss der Temperatur auf die Aktivität der mikrosomalen Oleyl-coenzym A Desaturase von Kartoffelknollen. Die Temperatur der Inkubation, zwischen 2 und 35 °C, ist in der Zeichnung angegeben.
Fig. 1. Effet de la température sur l'activité de l'oléyl-coenzyme A désaturase du tubercule de pomme de terre. La température d'incubation, variant de 2 à 35 °C, est indiquée sur le graphique.

temperatures. However, the level of the plateau reached after 30 min varies according to the temperatures, the highest level being observed at 30 °C. At low temperatures (2 and 10 °C), the desaturation is strongly reduced. At 10 °C, the slope of the curve measured for the first 15 min is nine times lower than at 30 °C; the plateau is observed after a longer time of incubation (90 min); the maximum activity is lower than at 20 °C. At 2 °C, the percentage of desaturation is almost zero; a low desaturation (about 7 %) is noticed after 90 min of incubation at this temperature.

These experiments show that the microsomal oleyl-coenzyme A desaturase of potato tuber presents its optimal activity at moderated temperatures and approximately follows classical enzymic kinetics. At low temperatures, although the solubility of oxygen in the incubation medium was greatly increased, oleyl-coenzyme A desaturase activity was low.

Fig. 2. Effect of oxygen on the activity of microsomal oleyl-coenzyme A desaturase of potato tubers, at 30 °C. The oxygen concentration of the incubation medium (varying from zero [N_2 = pure nitrogen] to 100 %) is indicated in the graph.



Time of incubation, min, Desaturation of . . .: *Siehe Abb. 1 - Voir fig. 1*

Abb. 2. Einfluss der Sauerstoffkonzentration auf die Aktivität der Oleyl-coenzym A Desaturase der Kartoffelknolle bei 30 °C. Die Sauerstoffkonzentration im Inkubationsmedium ist in der Zeichnung angegeben (zwischen 0 [reiner Stickstoff N_2] und 100 %).

Fig. 2. Effet de la concentration en oxygène sur l'activité de l'oléyl-coenzyme A désaturase du tubercule de pomme de terre, à 30 °C. La concentration en oxygène du milieu d'incubation (variant de zéro [N_2 = azote pur] à 100 %) est indiquée sur le graphique.

Effect of the oxygen concentration in the incubation medium

The desaturation kinetics of (1-^{14}C)-oleyl-coenzyme A in vitro (Fig. 2) at a fixed temperature (30 °C) and in atmospheres with various oxygen and nitrogen concentrations indicate a clear correlation between the desaturation activity and the oxygen concentration of the incubation medium. When the percentage of oxygen is increased from 5 to 20 % (this last approximating to normal air), a higher intensity of desaturation is observed for the first 30 min; a plateau is then obtained, with a highest level corresponding to 20 % of oxygen in the medium. In pure oxygen, some inhibition occurs, which may be due to the competing action of lipid-degrading enzymes (e.g. lipoxygenases), particularly active in cellular fractions prepared from potato tubers (Galliard, 1970).

The absolute requirement of aerobic conditions is indicated by the assays performed in pure nitrogen; no desaturation is observed in these conditions (Fig. 2).

Discussion

The oleyl-coenzyme A desaturase of the microsomal fraction of potato tuber requires for a high activity in vitro certain conditions of temperature and oxygen concentration.

The optimum temperature is around 30 °C; low temperatures considerably reduce the desaturation. Oxygen is a limiting factor for the desaburation, since this enzymatic activity is diminished by concentrations lower than 20% O_2.

The physiological interest of these results comes from this double requirement for a moderate temperature and of adequate aeration for an optimal activity of the oleyl-coenzyme A desaturase. These requirements are easily satisfied in vivo in a temperature climate.

Burton (1950) measured the oxygen in solution in the cell sap of potato tubers, stored in air at various temperatures and intact until the time of measurement, and found it to range from 3.5×10^{-4} mol/l at 1 °C to 2×10^{-5} mol/l at 37 °C. Relative to saturation at the same temperature with normal air, as opposed to intercellular oxygen concentration, the concentration ranged rom 93% at 1 °C to 10% at 37 °C, but only fell below 70% at temperatures of 20 °C and above.

These data therefore suggest that the hypothesis attributing to oxygen an essential role for the increase in the synthesis of polyunsaturated fatty acids at low temperatures is not applicable, at least within the potato tuber cell. It is probably more interesting to study what effects the low temperatures may have on the biogenesis of the oleyl-coenzyme A desaturase itself. It can be remembered that this enzyme is inducible in the potato tuber cell, particularly during the 'ageing' process (Abdelkader et al., 1973). It will be necessary, in order to explain the effect of low temperatures on the polyunsaturated fatty acid synthesis, to follow the effect of the temperature on the induction of the oleyl-coenzyme A desaturase.

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Zusammenfassung

Einfluss der Temperatur und des Sauerstoffes auf der Aktivität der mikrosomalen Oleyl-coenzym A Desaturase von Kartoffelknollen

Um zu prüfen, ob die Sauerstoffkonzentration in der Zelle - bei niedriger Temperatur höher - ein regulierender Faktor bei der Synthese der mehrfach ungesättigten Fettsäuren ist, wurde der Einfluss der Temperatur und der Sauerstoffkonzentration auf die Aktivität der mikrosomalen Oleyl-coenzym A Desaturase der Kartoffelknollen in vitro untersucht. Die höchste Aktivität des Enzyms wurde bei einer Temperatur von 30 °C gefunden und nicht bei tieferen Temperaturen (Abb. 1) und bei einer Konzentration von ca. 20 % Sauerstoff (Abb. 2). Diese Ergebnisse zeigen, dass die angeführte Hypothese im Falle der Kartoffelknollen nicht zutrifft.

Résumé

L'effét de la température et de l'oxygène sur l'activité de l'oléyl-coenzyme A désaturase microsomale du tubercule de pomme de terre

Pour vérifier si l'hypothèse selon laquelle la concentration en oxygène du milieu cellulaire - plus élevée à basse température - est un facteur régulant la synthèse des acides gras polyinsaturés, les effets de la température et de la concentration en oxygène sur l'activité de l'oléyl-coenzyme A désaturase microsomale du tubercule de pomme de terre ont été étudiés in vitro. L'activité la plus élevée de l'enzyme est observée pour la température de 30 °C et non à basse température (Fig. 1) et pour une concentration en oxygène d'environ 20 % (Fig. 2). Ces résultats suggèrent que l'hypothèse exposée plus haut n'est pas valable dans le cas du tubercule de pomme de terre.

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