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Thermodynamic parameters of enzymes in grassland soils from Galicia, NW Spain

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Abstract

The thermodynamic parameters of the enzymes catalase, dehydrogenase, casein-protease, α-N-benzoyl-L-argininamide (BAA)-protease, urease, Carboxymethyl (CM)-cellulase, invertase, β-glucosidase and arylsulphatase, were investigated in grassland soils from a European temperate-humid zone (Galicia, NW Spain). The effect of temperature on enzyme activity was determined at 5, 18, 27, 37, 57 and 70 °C. The temperature-dependence of the rate of substrate hydrolysis varied depending on the enzyme and soil. In general, the soil containing the least amount of organic matter (OM) showed the lowest enzyme activity for all temperatures and enzymes, whereas soils with similar OM contents showed similar levels of activity for the entire temperature range. Temperature had a noteworthy effect on the activity of oxidoreductases. Product formation in the reaction catalyzed by dehydrogenase increased with increasing temperature until 70 °C, which was attributed to chemical reduction of iodonitrotetrazolium violet (INT) at high temperatures. Catalase activity was not affected above 37 °C, which may be explained either by non-enzymatic decomposition of hydrogen peroxide or by the fact that catalase has reached *kinetic perfection,* and is therefore not saturated with substrate.

The Arrhenius equation was used to determine the activation energy (E_a) and the temperature coefficient (Q_{10}) for all enzymes. The values of E_a and Q_{10} for each enzyme differed among soils, although in general the differences were small, especially for those enzymes that act on substrates of low molecular weight. In terms of the values of E_a and Q_{10} and the differences established among soils, the results obtained for those enzymes that act on substrates of high molecular weight differed most from those corresponding to the other enzymes. Thus the lowest E_a and Q_{10} values corresponded to BAA-protease, and the highest values to CM-cellulase and casein-protease. Except for catalase in one of the soils, the values of E_a and Q_{10} for the oxidoreductases were similar to those of most of the hydrolases. In general, the effect of temperature appeared to be more dependent on the type of enzyme than on the characteristics of the soil.

Keywords: Soil enzymes; Hydrolases; Oxidoreductases; Thermodynamic parameters; Arrhenius equation; Activation energy; Temperature coefficient (Q_{10}); Climate change

1. Introduction

The rate of enzyme catalysis generally increases with increases in temperature until a critical point is reached when denaturation of the enzyme is initiated and the reaction rate begins to decrease (Skujins, 1967; Tabatabai, 1982). Thus, a peak in enyzme activity is observed, which is usually referred to as the optimum temperature and which varies for different enzymes. Most reports on the effect of temperature on soil enzyme activities refer to hydrolases and little is known about the temperature sensitivity of soil oxidoreductases. In the case of soil hydrolytic enzymes, the optimum temperature is usually between 10 and 15 °C higher than for enzymes in solution (Skujins, 1967; Tabatabai, 1982). The higher denaturation temperature for soil hydrolytic enzymes than for enzymes in solution is attributed to the fact that the former are usually protected by humic and clay colloids (Skujins, 1976; Burns, 1982), which results in greater stability against factors such as high temperatures, proteolysis, etc. (Nannipieri et al., 1978,

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1980). The temperature at which the activity of soil hydrolytic enzymes is maximal is usually between 50 and 60 °C (Tabatabai and Bremner, 1970; Ladd and Butler, 1972; Schinner and von Mersi, 1990; Frankenberger and Tabatabai, 1991a). However there are exceptions, e.g. the activity of aspartase decreases sharply above 40 °C (Senwo and Tabatabai, 1996) and phosphodiesterase activity is maximal at 70 °C (Browman and Tabatabai, 1978; Killman and Rashid, 1986).

Determination of the activity of an enzyme at different temperatures not only provides the optimal range of temperature for enzyme activity, but also allows estimation of thermodynamic parameters, e.g. the activation energy (E_a). The E_a for enzyme-catalyzed reactions is lower than for reactions not catalyzed by enzymes, because enzymes act by lowering the energy barrier that must be surmounted before the reaction can take place (Juma and Tabatabai, 1988). Determination of enzyme activity at different temperatures also allows the value of the temperature coefficient, or Q_{10}, to be obtained. This coefficient indicates the increase in the rate of reaction for increases in temperature of 10 °C. Enzyme-catalyzed reactions are less sensitive to temperature changes than their uncatalyzed counterparts. Thus, although the uncatalyzed reaction rate may double for every increase of 10 °C, the enzyme-catalyzed reaction rate generally increases by a factor of less than two (Tabatabai, 1982). The value of Q_{10} is not constant, and varies for different enzymes depending on the activation energy and on the temperature at which it is obtained (Lehninger, 1978).

There are many reports of the effects of incubation temperature on the activity of hydrolytic enzymes, although most of these studies have involved finding out the optimum temperature for activity (Geller and Ginzburg, 1979; Kanazawa and Miyashita, 1986; Trevors, 1984a; Kandeler, 1990; Schinner and von Mersi, 1990; Nannipieri et al., 1991). Furthermore, most published studies on the effect of the incubation temperature on the activity of soil enzymes refer to hydrolytic enzymes, and very few are concerned with oxidoreductase enzymes such as dehydrogenase or catalase. In any case, studies on thermodynamic parameters are very scarce and in fact, for many soil enzymes no studies of their thermodynamic parameters have been carried out. This is surprising considering the importance of these parameters, which are intrinsic characteristics of soil enzymes under any particular set of environmental conditions. Most of the published data on thermodynamic parameters originate from the members of one research group who, as well as developing methods for the determination of the activity of diverse soil enzymes, also characterized these enzymes by estimating their thermodynamic parameters. (e.g. Browman and Tabatabai, 1978; Juma and Tabatabai, 1988; Frankenberger and Tabatabai, 1991a, b; Senwo and Tabatabai, 1996). Although other researchers have also determined the thermodynamic parameters of soil enzymes, only a limited number of enzymes have been studied, including phosphomonoesterases (Tena-Aldave et al., 1979; Trasar-Cepeda and Gil-Sotres, 1988), ureases (Gould et al., 1973; Dalal, 1975; Kumar and Wagenet, 1984) and arylsulphatases (Perucci and Scarponi, 1984). Moreover, to our knowledge, there are no published studies of the thermodynamic parameters of a large number of soil enzymes in the same set of soils.

The lack of information on the relationship between temperature and enzyme activities is also surprising considering the current concern about the effects of climate change on the processes affecting organic matter (OM) mineralization and greenhouse gas emissions and the essential role of soil enzymes in these processes (Kirschbaum, 2004; Knorr et al., 2005). It is therefore evident that models for predicting the effects of climate change on decomposition should take into account the thermodynamic parameters characterizing soil enzymes.

In the present study, the effect of temperature on the activities of catalase, dehydrogenase and various hydrolases of the carbon, nitrogen and sulphur cycles was investigated in grassland soils from Galicia (NW Spain), a region situated north of 40°N, within a zone where the effects of climate change are considered to be particularly important (Lloyd and Taylor, 1994). The aim of the study was to determine the activation energy (E_a) and temperature coefficient (Q_{10}) for catalase, dehydrogenase, casein-protease, BAA-protease, urease, CM-cellulase, invertase, β-glucosidase and arylsulphatase.

2. Materials and methods

2.1. Soils

Three grassland soils (Navia, Sobrado, Ponteareas: **N**, **S** and **P**, respectively) developed over different parent material and under different climatic conditions, were selected for study (Table 1). The three soils were Umbrisols (ISSS Working Group RB, 1998) and the vegetation was a mixture mainly composed of ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.). At each site,

Table 1
Main characteristics of the three soils studied

	Navia	Sobrado	Ponteareas
Parent material	Slates	Basic schists	Granites
Temperature (°C)[a]	11	12	15
Annual rainfall (mm)	1453	1428	1543
pH in water	4.9	5.6	5.2
pH in KCl	3.9	4.6	4.3
Total C (%)	6.9	6.2	3.3
Total N (%)	0.61	0.41	0.19
C/N	11	15	17
Sand (%)	39	52	49
Silt (%)	43	33	26
Clay (%)	18	15	25
Texture	Loam	Sandy loam	Sandy clay loam

[a]Mean annual temperature.