Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Biotechnology 116 (2005) 1–10
JOURNAL OF Biotechnology
www.elsevier.com/locate/jbiotec

Improved stabilization of chemically aminated enzymes via multipoint covalent attachment on glyoxyl supports

Fernando López-Gallego, Tamara Montes, Manuel Fuentes, Noelia Alonso, Valeria Grazu, Lorena Betancor, Jose M. Guisán[1], Roberto Fernández-Lafuente*

Departamento de Biocatálisis, Instituto de Catálisis, CSIC, Campus Universidad Autonoma, Cantoblanco, 28049 Madrid, Spain

Received 28 May 2004; received in revised form 14 September 2004; accepted 24 September 2004

Abstract

The surface carboxylic groups of penicillin G acylase and glutaryl acylase were chemically aminated in a controlled way by reaction with ethylenediamine via the 1-ethyl-3-(dimethylamino-propyl) carbodiimide coupling method. Then, both proteins were immobilized on glyoxyl agarose.

In both cases, the immobilization of the chemically modified enzymes improved the enzyme stability compared to the stability of the immobilized but non-modified enzyme (by a four-fold factor in the case of PGA and a 20-fold factor in the case of GA). The chemical modification presented a deleterious effect on soluble enzyme stability. Therefore, the improved stability should be related to a higher multipoint covalent attachment, involving both the lysine amino groups and also the new amino groups chemically introduced on the enzyme. Moreover, the lower pK_a of the new amino groups permitted to immobilize the enzyme under milder conditions. In fact, the aminated proteins could be immobilized even at pH 9, while the non-modified enzymes could only be immobilized at pH over 10.

Keywords: Improved stabilization; Glyoxyl supports; Penicillin G acyclase

Abbreviations: PGA, penicillin G acylase from *E. coli*; GA, glutaryl acylase from *Pseudomonas* sp.; EDAC, 1-ethyl-3-(dimethylamino-propyl) carbodiimide; EDA, ethylenediamine; iPGA 4.75-2, immobilized PGA modified with 10^{-2} M EDAC at pH 4.75; iPGA 4.75-3, immobilized PGA modified with 10^{-3} M EDAC at pH 4.75; iGA 6-2, immobilized GA modified with 10^{-2} M EDAC at pH 6; PGA 4.75-3, PGA modified with 10^{-3} M EDAC at pH 4.75; GA 6-2, GA modified with 10^{-2} M EDAC at pH 6

* Corresponding author. Tel.: +34 91 585 48 09; fax: +34 91 585 47 60.
E-mail addresses: jmguisan@icp.csic (J.M. Guisán), rfl@icp.csic.es (R. Fernández-Lafuente).
[1] Co-corresponding author.

0168-1656/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jbiotec.2004.09.015

1. Introduction

The improvement of enzyme stability is still nowadays one of the main issues for the implementation of enzymes as industrial biocatalysts (Haki and Rakshit, 2003; Wong and Wong, 1992). Inactivation of enzymes usually starts with reversible conformational changes that finally promote their irreversible inactivation (Klibanov, 1983). Thus, most of the strategies for protein stabilization are focused on the prevention of the first three dimensional distortion of the protein. Among the available tools to solve this problem, we can highlight the search of enzymes from thermophiles (Adams and Kelly, 1998), protein engineering (Shaw and Day, 1999; Bryan, 2000; Svendsen, 2000), chemical modification (e.g., to introduce inter-protein cross-linkings) (Wong and Wong, 1992; Saidel et al., 1964; Tochilin et al., 1982) or the multipoint covalent immobilization (Martinek et al., 1977; Blanco et al., 1989). These tools are normally used in an individual and parallel way to improve enzyme stability (Gupta, 1991). However, in some cases the integrated use of several of these techniques may produce very interesting results. For example, the chemical modification of immobilized enzymes allows inter-protein or inter-subunit cross-linkages (Fernández-Lafuente et al., 1992, 1995, 2001) leading to enzyme derivatives with their tertiary and/or quaternary structures stabilized.

Chemical modification of proteins to improve their stability may be used following different strategies, mainly bearing in mind that most of industrial enzymes will be used in an immobilized form in biotransformations.

Usually, the researcher will intend to produce a modified enzyme with better stability than the unmodified enzyme. This improved enzyme will then be immobilized in a similar way that the unmodified enzyme, keeping its improved properties (Fig. 1).

The alternative proposed in this paper is to chemically modify the enzyme, not to get a more stable enzyme, but to have a protein surface enriched in reactive groups and, therefore, with much better possibilities of yielding a more intense multipoint covalent attachment during immobilization (Fig. 1).

Evidently, it is necessary to use a support that may really yield a very intense multipoint covalent attachment. Thus, to exemplify the potential of this strategy, the immobilization of enzymes on glyoxyl agarose was

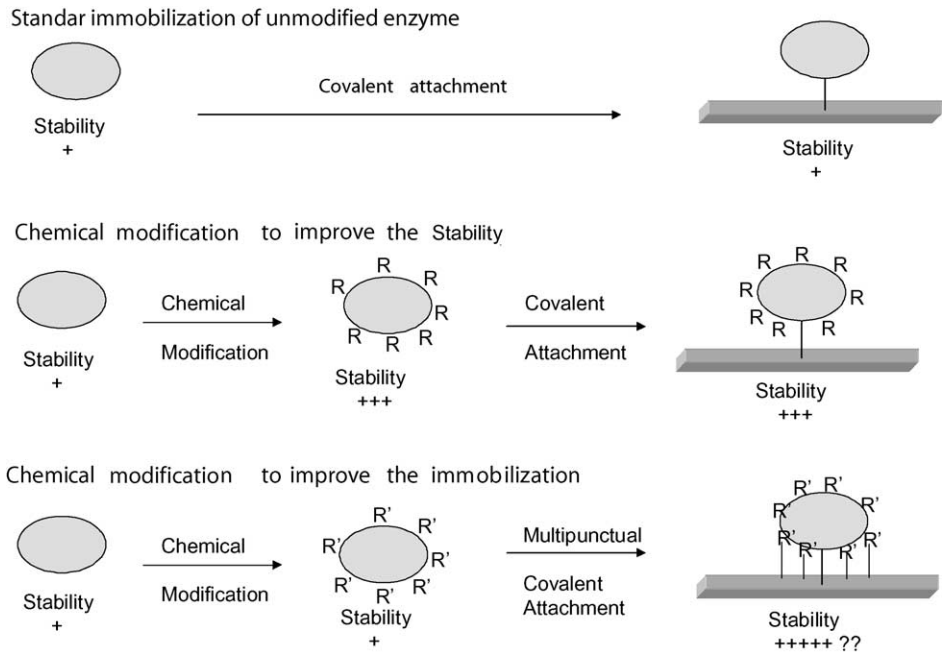

Fig. 1. Chemical modification for achieving a more stable immobilized preparation.