HIGH SENSITIVITY ON-GEL-DETECTION OF UNMODIFIED ELECTROPHORESED PROTEINS FOR SUBSEQUENT MICRO-ANALYSIS: THE PROTEIN REVERSE STAINING

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INTRODUCTION

Since the first report on reverse staining of SDS-PAGE gels by using imidazole-zinc salts (1), a very reproducible and sensitive modification of the zinc chloride stain (2); we have been concerned with its development in three main directions: (i) the extension of the reverse staining technique to detect proteins electrophoresed in absence of detergents or in presence of detergents other than SDS (e.g., Triton) e.g., on native or isoelectric-focussing polyacrylamide or agarose gels (3, 4); (ii) the efficient recovery of the detected proteins from gel for subsequent microanalysis, by electrotransfer onto PVDF membrane (5), onto reversed phase high performance liquid chromatography support (6) and electroelution (7); (iii) the development of a double staining technique to visualize Coomassie blue undetected proteins by using reverse staining as the second step in the double staining strategy (8).

METHODS

Reverse-staining of gels was performed as reported (1, 3, 4) whereas double-staining of Coomassie blue stained gels was described in (8). Mobilization solutions contained 200 mM glycine, or 100 mM dithiothreitol or 50 mM EDTA, pH 8.3 (5).

RESULTS AND DISCUSSION

We have gained structural information from polyacrylamide-gel-electrophoresed proteins after on-gel detection by imidazole-SDS-zinc reverse staining. As a consequence of reverse staining: a) protein bands arise transparent against a deep white stained bakground, limits of detection being in the femtomol range (10 to 1 ng protein per band); b) there is no loss of image when the gel is kept in distilled water (even during years); c) protein bands result immobilized i.e., they do not diffuse upon gel storage. To recover reverse stained proteins or fragments thereof from gel, the immobilization of bands must be first abrogated by chelating the zinc ions from stain (protein mobilization). Proteins could be mobilized, at any time after staining, by short term (10 to 5 min) incubation of the gel in solutions of glycine, dithiothreitol, 2-mercaptoethanol, at neutral to alkaline pH, or of EDTA, at alkaline pH. Thus mobilized proteins were amenable to electroblotting and analysis, by N-terminal sequencing or deblocking (on-PVDF), or enzymatic or chemical cleavage (on-gel or on-PVDF), or Western-blotting, as efficiently as they were unstained. We have further developed a new double staining of gels already-stained with Coomassie-blue, by using imidazole-SDS-zinc reverse staining, for (a) detecting CB-undetectable proteins on-gels and (b) circumventing disadvantages of those double-stains that use silver-technique as the second step in the double-staining strategy. As a result, a homogeneous white-stained background is generated and two types of protein bands can be observed: (a) typical CB-stained bands which appear supperpossed on larger transparent bands, and (b) reverse-stained transparent bands. Proteins are not chemically modified by this new technique, overall sensitivity being as high as that of reverse staining. We believe that a further development of the present methodology should allow its extension to routine femtomolar protein analysis.

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