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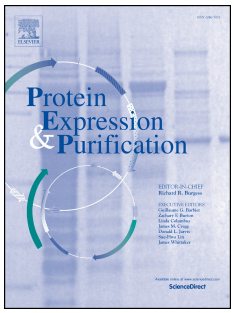

Improved high-yield expression, purification and refolding of recombinant mammalian prion proteins under aerosol-free elevated biological safety conditions

Peter Rehbein, Harald Schwalbe

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Improved high-yield expression, purification and refolding of recombinant mammalian prion proteins under aerosol-free elevated biological safety conditions

Peter Rehbein* and Harald Schwalbe*

Institute for Organic Chemistry and Chemical Biology, Center of Biomolecular Magnetic Resonance, Goethe University Frankfurt, Max-von-Laue-Strasse 7, 60438 Frankfurt am Main, Germany

*Corresponding authors: Peter Rehbein and Harald Schwalbe, Institute for Organic Chemistry and Chemical Biology, Center of Biomolecular Magnetic Resonance, Goethe University Frankfurt, Max-von-Laue-Straße 7, 60438 Frankfurt am Main, Germany
email: schwalbe@nmr.uni-frankfurt.de; fax: ++49-69-29515.

ABSTRACT

Production of recombinant prion proteins is of crucial relevance in food technology (analytical standards, assay development) but also in basic research, most importantly structural biology (NMR, X-ray diffraction). Structural approaches conveniently allow for sophisticated investigation of prion disease pathogenesis, but usually require large amounts of sample material. Recently, working with recombinant prion proteins has been recategorized to biosafety levels > S1 as infectious prions may readily be generated *de novo* and become airborne *via* aerosols. Heterologous expression should therefore be established with appropriately adjusted safety precautions. We have developed a protocol for high-yield expression, purification and refolding of recombinant mammalian prion proteins at elevated biological safety levels by introducing means of abolishing aerosol formation and propagation.

Keywords: prion, biological safety level, protein, expression, purification, refolding

Abbreviations:
PrP^{C} - major prion protein of higher animals (cellular form), PrP^{SC} (major prion protein of higher animals (scrapie form), SDS-PAGE - sodium dodecyl sulfate polyacrylamide gel electrophoresis, DMAPS - 3-(N,N-Dimethyloctylammonio)propanesulfonate, C7BzO - 3-(4-Heptyl)phenyl-3-hydroxypropyl)dimethylammoniopropanesulfonate

INTRODUCTION

Infectious neurodegenerative disorders including bovine spongiformous encephalopathy (BSE) in cattle or Creutzfeldt-Jakob disease (CJD) in humans are caused by a misfolded isoform of the major prion protein (PrP), which may form a proteinaceous infectious particle ("prion") [1, 2]. While retaining the primary structure, PrP is able to adopt two distinct tertiary structures: The native, cellular state (PrP^{C}), which features a high content of alpha-helical secondary structure [3], and a misfolded, aggregation-prone abnormal isoform (PrP^{Sc}), which mainly consists of beta-sheet secondary structure [4]. Infectivity of prion-diseases is conveyed by the fascinating, yet disastrous fact that PrP^{Sc} is able to imprint its misfolded conformation to monomers of PrP^{C} on contact in an autocatalytic manner [5]. In addition, PrP^{Sc} is highly insoluble and thus forms populations of oligomeric assemblies of yet unknown structure. These reorganize into fibrillar superstructures designated as amyloids, which due to their size, evade intracellular proteolysis and eventually cause neuronal necrosis [6].