

Important Factors Influencing Protein Solubility for 2-D Electrophoresis

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Fig. 1. Example of a 2-D gel section exhibiting poor protein solubilization.

Protein solubility is a critical factor for successful 2-D gel electrophoresis; a good result requires that the proteins to be separated be well solubilized prior to and throughout the separation procedure.

Protein solubilization is the process of breaking interactions involved in protein aggregation (Rabilloud 1996), which include disulfide bonds, hydrogen bonds, van der Waals forces, ionic interactions, and hydrophobic interactions. If these interactions are not prevented, proteins can aggregate or precipitate, resulting in artifacts or sample loss (see Figure 1).

This article describes factors that affect protein solubility and ways to improve it to ensure better 2-D results. The measures taken to ensure solubility at the stage of sample preparation and electrophoresis can be different, so the issues of solubility during these two stages of the procedure will be considered separately.

Solubility Factors Associated With Sample Preparation

Proteins in a biological sample are generally present in their native state. They are often associated with other proteins as well as integrated into membranes or are part of large complexes. In many cases they are not soluble in their native state after isolation and must be denatured to help solubilization.

Effective sample preparation for 2-D electrophoresis disrupts these associations and solubilizes as large a subset of the protein population as possible. Sample solubilization solutions typically contain a number of compounds including chaotropic agents, detergents, reducing agents, buffers, salts, and ampholytes. These additives are discussed separately.

There are instances in which a deliberate choice may be made to solubilize only a subset of the proteins in a sample. This may be done to reduce sample complexity, or if solubility conditions for the proteins of interest have already been determined. ReadyPrep™ protein extraction kits operate on the principle of selective inclusion or omission of different solubilizing agents to accomplish sample fractionation. For example, the ReadyPrep sequential extraction kit (catalog #163-2100) is based on the methods described by Molloy et al. (1998) for the selective solubilization of different protein pools.

Chaotropic Agents

These compounds disrupt hydrogen bonds and hydrophobic interactions both between and within

proteins. When used at high concentrations, chaotropic agents disrupt secondary protein structure and bring into solution proteins that are not otherwise soluble. The neutral chaotropic agent urea is used at concentrations from 5 to 9 M, often with thiourea at concentrations up to 2 M. Thiourea can dramatically increase the number of proteins solubilized (Rabilloud et al. 1997), so this additive is used when otherwise insoluble proteins are to be separated. If thiourea is used during sample preparation, it should also be present in the first-dimension rehydration solution; otherwise, the proteins that require thiourea for solubility will come out of solution during IEF. Urea and thiourea can hydrolyze to cyanate and thiocyanate, respectively, which can modify amino groups on proteins and give rise to artifactual charge heterogeneity. Hydrolysis of urea and thiourea is promoted by heat, so care must be exercised to ensure that urea solutions are not heated in the presence of protein. Charged chaotropic agents such as guanidine hydrochloride are not used because they are incompatible with subsequent IEF. Chaotropic agents and detergents (see below) should both be used in sample preparation to enhance solubility. How much of each needs to be determined empirically.

Detergents

These compounds also disrupt hydrophobic interactions between and within proteins and are particularly useful in solubilizing otherwise insoluble proteins. They are typically used at concentrations of 1–4%. The variety among detergents is huge, with hundreds available commercially. Detergents are classified according to their charge in solution. They may be anionic, cationic, neutral, or zwitterionic. Sample preparation for 2-D electrophoresis commonly uses neutral or zwitterionic detergents, because they are most compatible with subsequent IEF separation. Examples of neutral detergents include octyl glucoside, dodecyl maltoside, and Triton X-100. Examples of zwitterionic detergents include CHAPS, CHAPSO, and ASB-14. The anionic detergent SDS is also commonly used during sample preparation for 2-D electrophoresis. SDS is unparalleled in its ability to solubilize proteins efficiently and rapidly. However, since it is charged and forms tight associations with proteins, precautions must be taken to ensure that SDS does not interfere with IEF separation. The best detergent to use in any given situation is hard to predict and is best determined empirically.

Reducing Agents

Reducing agents are also commonly used during sample preparation to cleave disulfide bond crosslinks within proteins and between protein subunits. The compounds used for 2-D sample preparation are either sulfhydryl or phosphine reducing agents. Examples of sulfhydryl reductants include dithiothreitol (DTT), dithioerythritol (DTE), and β -mercaptoethanol. The first two can be used at lower concentrations and are more commonly used. Examples of phosphine reductants include tributylphosphine (TBP) and tris-carboxyethylphosphine (TCEP). These reducing agents can be used at lower concentrations than the sulfhydryl reductants and are active over a wider pH range. Alkylation of a sample after treatment with a reducing agent can be done at the sample preparation stage as well. The treatment blocks protein sulfhydryls and prevents proteins from aggregating and precipitating due to oxidative crosslinking (see Solubility Factors Associated With Electrophoresis). This ensures that proteins remain soluble throughout electrophoresis. The ReadyPrep reduction-alkylation kit (catalog #163-2090) is used for this purpose.

Buffers, Salts, and Ampholytes

The pH and ionic strength of the solution both have a strong influence on protein solubility, so buffers, ampholytes, and salts are commonly added to sample preparation solutions. Many proteins are optimally soluble at high pH, so Tris base is often included to elevate the pH. However, different proteins are soluble at different pH values, so the use of a different buffer can result in a different set of proteins being extracted. The choice of buffer and pH of the sample preparation solution can therefore have a strong influence on the proteins that show up in the 2-D pattern. Some proteins require ionic interactions for solubility, so salt solutions (for example, 150 mM NaCl) are often used for protein extraction. The use of salt requires caution because of its disruptive effect on IEF; it can be removed with the ReadyPrep 2-D cleanup kit (catalog #163-2130). Carrier ampholyte mixtures are a common addition to sample preparation solutions because they increase both buffering power and ionic strength without subsequently interfering with IEF.

Denaturation and solubilization can require some time to complete. It might therefore be necessary to apply solubilization treatments for up to several hours before the sample is ready for IEF. Solubilization may be accelerated by sonicating the sample, but care must be taken that this treatment does not result in heating, particularly if the solubilization solution contains urea. Samples should be sonicated with bursts lasting no longer than several seconds, with cooling on ice between bursts.

In some cases, the presence of nonprotein impurities may limit protein solubility. Proteins may form complexes with lipids or nucleic acids that prevent their full solubilization. In these cases, it can

be advantageous to remove nonprotein impurities with a cleanup procedure such as TCA-acetone precipitation (Damerval et al. 1986) or treatment with the ReadyPrep 2-D cleanup kit (see Figure 2). In many cases, sample extraction and solubilization can be done with the sample solution used for rehydration prior to IEF. This approach is preferable, since it simplifies the procedure and ensures that no IEF-incompatible components are introduced during sample preparation.

Solubility Factors Associated With Electrophoresis

Proteins that have been solubilized during sample preparation must remain soluble during subsequent electrophoresis steps. This is particularly critical during the first-dimension IEF step, where problems of protein aggregation and precipitation most often occur. Solubility-related artifacts are less of a problem during the second-dimension SDS-PAGE step. This section therefore concentrates on the measures taken to ensure protein solubility during first-dimension IEF.

Many of the solubilizing agents used during sample preparation are also used during first-dimension IEF, but the choices are more limited because they must be compatible with IEF. Any compound present in the rehydration solution should be uncharged or weakly charged. The concentration of any charged compounds that were used during sample preparation (for example, buffers, salts, charged detergents) should be reduced, either by substantial dilution into rehydration solution, or by removal using dialysis or treatment with the ReadyPrep 2-D cleanup kit. A chaotropic agent, detergent, reducing agent, and carrier ampholytes are almost always added to the IEF rehydration solution.

Chaotropic Agents

Urea is always used in the IEF rehydration solution. If urea is the only chaotropic agent used, it should be at a concentration of at least 8 M. When used with thiourea, it should be at a concentration of at least 7 M. Thiourea may be used at concentrations up to 2 M. Thiourea can, however, cause disturbances in the 2-D pattern at the acidic end of the pH gradient. This tendency must be balanced against the desire to have more proteins represented in the final 2-D pattern when choosing whether or not to use this reagent in sample preparation and first-dimension IEF.

Detergents

The detergent used during IEF must be either neutral or zwitterionic. Zwitterionic detergents generally provide better solubilizing power (Chevallet et al. 1998, Perdew et al. 1983), although there are some exceptions (Luche et al. 2003). CHAPS is the most commonly used detergent and can be used at concentrations up to 4%. It is the safest choice due to its high solubility and relative lack of detergent-induced artifacts in 2-D results.

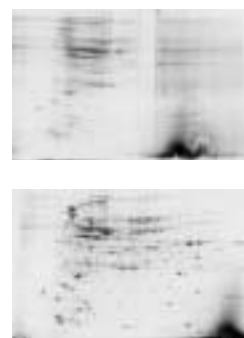


Fig. 2. Removal of even very high salt concentrations from samples using the ReadyPrep 2-D cleanup kit. *E. coli* extracts containing 1 M NaCl before (top) and after (bottom) removal of salts.

Alkylsulfobetaines such as SB 3-10 have also been used. These detergents are relatively insoluble in urea solutions and require urea concentrations of 5 M or less. Amidoalkylsulfobetaines such as ASB14 are compatible with high concentrations of urea and thiourea and are reported to have superior solubilizing properties (Chevallet et al. 1998). The effects of detergents are generally not additive and can be unpredictable in mixtures, so care should be taken when using more than one detergent in a rehydration solution.

Reducing Agents

Unfortunately, none of the commonly used reducing agents are uncharged over the entire pH range used for IEF. DTT is an adequate reductant for IEF in a range below pH 8. However, oxidative crosslinking is promoted at high pH, and there is no good reductant for proteins that is active above pH 8 (Görg et al. 2000, Hoving et al. 2002). If proteins are to be resolved above pH 8, this problem should be avoided by reducing and alkylating the sample with the ReadyPrep reduction-alkylation kit (Brubacher et al. 2003) prior to the first-dimension run.

Ampholytes

The presence of carrier ampholytes (such as Bio-Lyte® ampholytes) is required to maintain solubility during IEF. They function by preventing proteins from interacting with each other ionically, and do so without disturbing the pH gradient or interfering with electrophoresis.

The ability to manipulate proteins throughout the process of 2-D electrophoresis is dependent on the

protein remaining soluble during sample preparation as well as electrophoresis. This article presents important factors that affect protein solubility and ways to improve 2-D results. If you have further questions regarding protein sample solubility or any other technical issues, please contact our technical support team at consult.bio-rad.com

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