ELSEVIER

Journal of Chromatography B, 691 (1997) 192–196

JOURNAL OF CHROMATOGRAPHY B

Short communication

Reduction of wall adsorption in capillary zone electrophoresis of a basic single-chain antibody fragment by a cationic polymeric buffer additive

M. Morand[a], D. Blaas[b], E. Kenndler[a,*]

[a]*Institute of Analytical Chemistry, University of Vienna, Währingerstrasse 38, A-1090 Vienna, Austria*
[b]*Institute of Biochemistry, Medical Faculty, University of Vienna, Dr. Bohrgasse 3, A-1030 Vienna, Austria*

Received 9 April 1996; revised 6 August 1996; accepted 7 August 1996

Abstract

Reduction of adsorptive protein–wall interactions by poly(diallyldimethyl ammonium chloride), a permanently cationic polymer, at a concentration of 0.5% (w/v) is demonstrated for a basic single-chain antibody fragment (scFv, p*I* about 9.5) even in the range of physiological pH of around 7. The polymer additive forms a positively charged layer at the silica surface which reverses electroosmosis and leads to electrostatic repulsion of the positively charged basic protein.

Keywords: Proteins; Single-chain antibody fragments

1. Introduction

The rather time-consuming and laborious hybridoma technique introduced by Kohler and Milstein [1] for the production of monoclonal antibodies has recently been complemented by a methodology based on filamentous phage libraries with each individual phage displaying a different single-chain antibody fragment on its surface. Antigen is immobilized on a suitable carrier, incubated with the phage library and those phages which are specifically bound are subsequently eluted. Repetition of this selection step leads to enrichment of phage displaying high affinity antibodies. After cloning, individual soluble antibody fragments are then obtained upon infection of a suitable *Escherischia coli* strain [2].

Not only are these antibody fragments easily obtained, they also have the considerable advantage of being smaller than conventional antibodies, being only monovalent and not causing problems associated with the presence of the Fc-portion in normal immunoglobulins. Single-chain antibody fragments (scFvs) with a tremendous number of different amino acid sequences in their complementarity determining regions (CDRs) are thus available. As a consequence, most of them are expected to exhibit different charges making capillary electrophoresis (CE) potentially suitable for their separation.

However, as found for many proteins, CE is often limited by disturbances due to adsorptive interactions with the wall of the fused-silica capillary, leading to asymmetric peaks and loss of resolution.

To overcome these interferences, strategies to supress adsorption have been described including either dynamic or permanent coating of the surface, or addition of substances to the buffer which com-

*Corresponding author

PII S0378-4347(96)00385-4

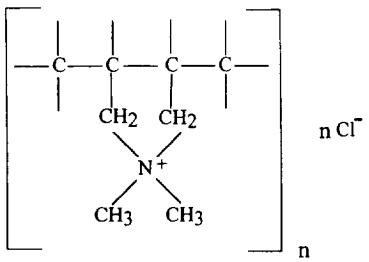

Fig. 1. Structural formula of poly(diallyldimethylammonium chloride) used as cationic additive.

pete with the proteins for the active sites of the capillary [3–16].

In the present paper the suitability of the recently introduced permanently cationic polymer poly(diallydimethylammonium chloride) (PDADMAC) [17], with the structural formula shown in Fig. 1, is investigated for the reduction of adsorptive interactions. The basic single-chain antibody fragment scFv7 (with a p*I* of about 9.5, as calculated from the amino acid sequence), which was obtained by screening a phage library for affinity towards the chicken very low density lipoprotein receptor [18], was chosen as a model system. As analysis of complex formation between antibodies and their cognate antigens requires buffer systems in the physiological pH range, and scFv7 is positively charged under these conditions, it was anticipated that the strong electrostatic attraction to the bare silica surface would lead to extensive adsorption. We demonstrate here that PDADMAC substantially reduces these interactions and that clean peaks of scFv7 can be obtained even at the critical pH around 7.

2. Experimental

2.1. Chemicals

Boric and phosphoric acid (both reagent grade) for the preparation of the buffer were obtained from E. Merck (Darmstadt, Germany); sodium hydroxide (0.1 *M* and 1.0 *M* for HPCE); hydroxypropylmethyl cellulose (HPMC), hydroxyethyl cellulose (HEC), poly(ethylene glycol) (PEG), triethanolamine hydrochloride (p.a., min. 99.5%) and poly(diallyldimethylammonium chloride) (PDADMAC, low molecular mass, 20 w/v% in water) were from Sigma (St. Louis, MO, USA), hydrochloric acid (p.a., min. 35%) from Loba Chemie (Fischamend, Austria).

Water was purified with a Milli Q water purification system (Millipore, Bedford, MA, USA). ScFv7 (molecular mass about 27 000) was prepared at the Institute for Biochemistry (Vienna, Austria) and was used at concentrations of between 0.6 and 1.0 mg/ml (dissolved in phosphate buffer, 0.02 mol/l, pH 7.5).

2.2. Apparatus

All experiments were performed using an HP3D capillary electrophoresis system (Hewlett-Packard, Waldbronn, Germany), consisting of a CE unit with built-in diode array detector (DAD) and an HP3DCE ChemStation for system control, data collection and data analysis.

The instrument was equipped with uncoated fused-silica capillaries from Polymicro Technologies (Bloomfield, NJ, USA), 50 mm I.D. All experiments were carried out at 25.0°C.

2.3. Procedures

2.3.1. Buffer preparation

Boric acid (0.100 mol/l) and phosphoric acid (0.010 mol/l) were adjusted with NaOH (0.5 mol/l) to pH 7.0, 8.0, 9.0, 10.0 and 11.0, respectively. HPMC, HEC and PEG, (each 0.1%, w/v) was added to water (60°C) and dispersed for 5 min. Then the solution was shaken for about 6 h at ambient temperature. The clear solution was stored at room temperature and filtered (0.45-μm syringe filter, Millipore) prior to use.

The additive PDADMAC was dissolved in triethanolamine–HCl buffer (0.050 mol/l, pH 7.0) to give a 0.5% (w/v) solution.

2.3.2. Capillary treatment

New, bare fused-silica capillaries were cleaned by flushing with NaOH (1 mol/l) for 15 min and 0.1 mol/l for additional 15 min, followed by rinsing with water for 30 min. For PDADMAC coating the clean capillary was flushed with the PDADMAC solution (5 mg/ml) for 20 min.

2.3.3. Electrophoresis procedures

Before each run the capillary was flushed for 2 min with NaOH (0.1 mol/l), 1.5 min with water and 3 min with the running buffer. For the experiments with borate and phosphate buffer, and neutral additives, respectively, a capillary (32.5 cm total, 24 cm effective length) was used at a field strength of 460 V/cm. Under these conditions the EOF was directed towards the cathode. The sample was injected by pressure (500 Pa, 8 s). After changing the buffer additive the capillary was flushed for 15 min with NaOH (0.1 mol/l).

The experiments with triethanolamine–HCl buffer and PDADMAC as additive were carried out in a capillary (33.5 cm total, 25 cm effective length) at a field strength of 390 V/cm. Due to the cationic additive the EOF was directed towards the anode. The sample was injected by pressure (500 Pa, 3 s). There was no need to wash the capillary between the runs.

3. Results and discussion

Capillary zone electropherograms of the antibody fragment were measured at different pH values in solutions without and with non-ionic additives reported to reduce the adsorption of proteins onto the wall. Those without additive are shown in Fig. 2 for pH values of 7.0, 8.0, 9.0, 10.0 and 11.0. At pH 11 the protein exists mainly as anion (its p*I* is about 9.5) and should not be adsorbed significantly at the anionic silica surface. This is in fact what is observed experimentally. At pH 11 the protein peak is asymmetric and seems to tail. This is not necessarily caused by adsorption; it can be the result of microheterogeneities of the protein, leading to a number of poorly resolved peaks in addition to the main form. With decreasing pH the peak becomes smaller and is virtually absent at pH values below 9. This is caused by a decrease of the protein's negative net charge as it approaches its isoelectric point. The lower negative charge leads to an increase of (seemingly irreversible) adsorption, resulting in a smaller peak area of the protein. At pH 8, the protein is almost quantitatively adsorbed at the wall and delivers almost no noticable peak. Therefore, a further decrease of the pH towards physiological lower pH ranges is meaningless.

It was observed that electrically neutral polymers, as HPMC, HEC or PEG, often successful to reduce protein–wall interactions, fail for the case of the basic protein scFv7. The electrophoretic separation was clearly improved by the addition of these polymers to the buffer (results not shown). Loss of protein due to irreversible adsorption was found much less at pH 9 and 10 as compared to the buffer without additive. However, most of the protein

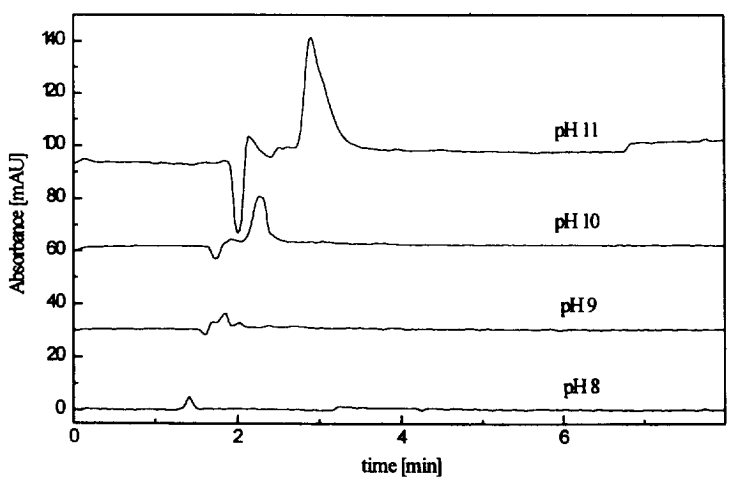

Fig. 2. Capillary zone electropherograms of scFv7 in borate buffer at different pH values without additive. Buffer concentration, 0.10 mol/l; capillary length, 32.5 cm (24 cm); voltage, +20 kV; UV absorbanc detector (195 nm) placed at the cathode side of the capillary. The negative jumps in the baseline at ca. 2 min indicate the EOF.

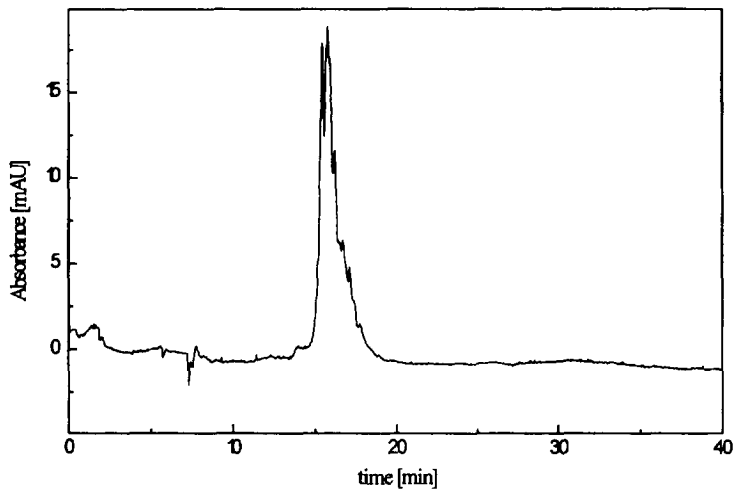

Fig. 3. Capillary zone electropherogram of scFv7 at pH 7.0 with PDADMAC as additive (concentration 0.5% w/v). Buffer, triethanolamine–HCl, 0.050 mol/l; capillary length 33.5 cm (25.0 cm); voltage, −13 kV; UV absorbance detector (200 nm) placed at the cathode side of the capillary. The EOF is indicated by the slight negative jump of the baseline at 7.5 min.

appears to be adsorbed at pH 8, and at pH 7 potential protein peaks could not be distinguished from baseline noise. This leads to the conclusion that the strong protein–wall interaction precludes the study of protein–ligand interactions by CZE in the physiological pH range even in the presence of nonionic additives.

Using the cationic polymer PDADMAC as additive most adsorptive interactions between the protein and the surface of the bare fused-silica surface were eliminated even at a pH as low as 7 (Fig. 3). Formation of a positively charged surface layer of the additive leads to reversal of the EOF, which is thus directed towards the anode. As a consequence, the protein, being positively charged at this pH migrates against the EOF to the anode. In contrast to the other systems the protein peak is clearly seen at pH 7 and even microheterogeneities of the protein are resolved. Comparison of the peak heights also indicates absence of adsorption.

4. Conclusion

From our results it can be concluded that PDADMAC is very effective in reducing adsorption of proteins to the capillary wall even in the case of basic proteins. The permanently cationic surfactant forms a layer with positive charges on the capillary surface, which reverses the EOF and leads to electrostatic repulsion of the protein. Further experiments aimed to determine the affinity constants of the interaction between scFv7 and its cognate antigen by methods similar to those described by Chu et al. [19] are currently under way.

Acknowledgments

This work was supported by the Austrian Science Foundation, Grant P-10384-MOB, to D.B.; the authors thank Doris Pfistermüller for the preparation of scFv7.

References

[1] G. Kohler and C. Milstein, Nature, 256 (1975) 495.
[2] A.D. Griffiths, Curr. Opinion Immunol., 5 (1993) 263.
[3] S.F.Y. Li, Capillary Electrophoresis, Principles, Practice and Applications, J. Chromatogr. Library, Vol. 52, Elsevier, Amsterdam, 1992.
[4] N.A. Guzman (Editor), Capillary Electrophoresis Technology, Marcel Dekker, New York, 1993.
[5] D. McManigill and S.A. Swedberg, in T. Hugli (Editor), Techniques in Protein Chemistry, Academic Press, San Diego, 1989.

[6] S. Hjerten, J. Chromatogr., 347 (1985) 191.
[7] Z. Zhao, A. Malik and M.L. Lee, Anal. Chem., 65 (1993) 2747.
[8] J.A. Bullock and L.C. Yuan, J. Microcolumn Sep., 3 (1991) 241.
[9] L. Emmer, M. Jansson and J. Roeraade, J. Chromatogr., 547 (1991) 544.
[10] G. Bruin, J.Chang, R. Kuhiman, K. Zegers, J. Kraak and H. Poppe, J. Chromatogr., 471 (1989) 429.
[11] J.K. Towns and F.E. Regnier, J. Chromatogr., 516 (1990) 69.
[12] J.K. Towns and F.E. Regnier, Anal. Chem., 63 (1991) 1126.
[13] M. Zhu, R. Rodriguez, D. Hansen and T. Wehr, J. Chromatogr., 516 (1990) 123.
[14] M. Minarik, B. Gas, A. Rizzi and E. Kenndler, J. Cap. Elec. 2 (1995) 89.
[15] B.M. Minarik, F. Groiss, B. Gas, D. Blaas and E. Kenndler, J. Chromatogr. A, 738 (1996) 123.
[16] J.-L. Liao, J. Abramson and S. Hjerten, J. Cap. Elec., 2 (1995) 191.
[17] N. Cohen and E. Grushka, J. Cap. Elec., 1 (1994) 112.
[18] Hodits, R.A., J. Nimpf, D.M. Pfistermueller, T. Hiesberger, W.J. Schneider, T.J. Vaughan, K.S. Johnson, M. Haumer, E. Kuechler, G. Winter and D. Blaas, J. Biol. Chem., 270 (1995) 24078.
[19] Y.H. Chu, W.J. Lees; A. Stassinopoulos and C.T. Walsh, Biochemistry, 33 (1994) 10616.