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Short Communication

Monoclonal gammopathy missed by capillary zone electrophoresis

Christof Schild[1,*], Florence Egger[1], Alain Kaelin-Lang[2] and Jean-Marc Nuoffer[1]

[1] Institute of Clinical Chemistry, Inselspital, Bern University Hospital, and University of Bern, Bern, Switzerland
[2] Department of Neurology, Inselspital, Bern University Hospital, Bern, Switzerland

Abstract

Background: Serum protein electrophoresis is used as a screening test for monoclonal gammopathies. Here, we present a case of a high-concentration monoclonal immunoglobulin (M-protein) that was missed by serum protein electrophoresis on a Capillarys 2 capillary zone electrophoresis system. The aim of our study was to identify the reason for the failure of the system to detect the M-protein.
Methods: M-protein solubility was examined in response to temperature, pH, ionic strength, the chaotropic agent urea and the reducing agent 2-mercaptoethanol.
Results: Precipitation of the M-protein was not cold-induced, but solubility decreased at pH 8.5 or higher, when the pH approached the apparent isoelectric point. The M-protein also precipitated in alkaline Capillarys 2 electrophoresis buffer (pH 10), which was the reason for the false-negative electrophoresis result. Precipitation of the M-protein was not related to the ionic strength of the buffer. Solubility improved in presence of urea. Pre-treatment of serum with 2-mercaptoethanol revealed the missing M-protein peak of 36 g/L on the electropherogram.
Conclusions: This case shows that insolubility of M-proteins in alkaline buffer is one possible cause of false-negative results on capillary zone electrophoresis systems. False-negative results should be considered, especially when accompanying laboratory results are inconsistent with the electropherogram.

Keywords: capillary zone electrophoresis; gammopathy; monoclonal components; monoclonal proteins.

*Corresponding author: Christof Schild, Institute of Clinical Chemistry, Inselspital, 3010 Bern, Switzerland
Fax: +41-31-632-48-62, E-mail: christof.schild@insel.ch
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Serum protein electrophoresis is recommended as part of the screening panels for the detection of monoclonal gammopathies, and is further used in the monitoring of the patients (1–4). Monoclonal immunoglobulins (M-proteins) from patients with monoclonal gammopathies exhibit individual physicochemical properties. Certain properties may cause clinical symptoms (e.g., polyneuropathies, primary amyloidosis) (3), interfere with the laboratory evaluation of the M-protein itself (5–9) or interfere with other laboratory assays (10–12).

Here, we report a case of an IgMκ M-protein insoluble in alkaline pH, which could not be detected by capillary zone electrophoresis using the Capillarys 2 (Sebia, Issy-les-Moulinaux, France). The protein electrophoresis of a patient serum showed normal fractions (%) with only a minor irregularity in the β region of the electropherogram (Figure 1A). However, an elevated total serum protein of 104 g/L (by biuret method, Modular P800, Roche Diagnostics, Rotkreuz, Switzerland) as well as a highly elevated total IgM of 62 g/L (by turbidimetry, Modular P800, Roche Diagnostics, Rotkreuz, Switzerland) were in contradiction with the almost normal serum protein electrophoresis. Immunofixation on Hydragel gels (Sebia, Issy-les-Moulinaux, France) finally revealed a distinctive IgMκ monoclonal protein. Closer examination of the dilution segment of the Capillarys 2 capillary electrophoresis analyzer revealed a precipitate. This precipitate consisted mainly of the missing monoclonal IgMκ, as proven by immunofixation of the precipitate previously solubilised in 30% Fluidil (Sebia, Issy-les-Moulinaux, France), 1.5% 2-mercaptoethanol for 15 min (data not shown). Retrospectively, the patient's serum was measured five times using the Capillarys 2, and always showed an electropherogram similar to Figure 1A, without a monoclonal peak. However, in two out of five runs, the maximal optical density of the albumin peak was below the specification of the manufacturer, which might indicate insufficient sampling into the capillary either due to increased viscosity or partial blocking of the capillary.

The insolubility of the monoclonal IgMκ was not cold-induced because the patient serum could be stored for weeks at 4°C without any signs of cryoglobulin precipitation. Instead, the alkaline pH of the Capillarys 2 electrophoresis buffer (protein 6 buffer, Sebia, Issy-les-Moulinaux, France) consisting of borate pH 10 plus additives was found to be the cause of the insolubility. Two lines of evidence support the insolubility in alkaline buffer: first, the precipitate could be solubilised in Capillarys 2 electrophoresis buffer that was neutralised to pH 7 (not shown). Second, the pH-dependent precipitation was also observed when a different buffer sys-

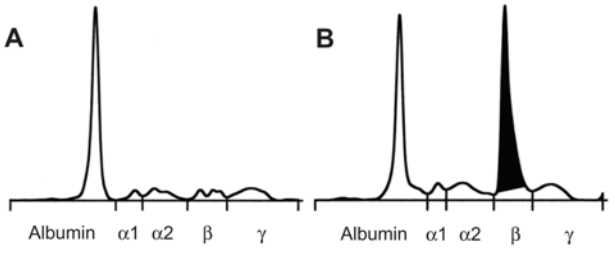

Figure 1 Electropherogram of the patient's serum before and after treatment with 2-mercaptoethanol.
Native (A) or 2-mercaptoethanol treated patient serum (B) were analyzed by capillary zone electrophoresis on Capillarys 2 [protein(e)6 buffer, software version 6.1.2]. For 2-mercaptoethanol treatment, 200 μL serum was incubated with 100 μL 5% 2-mercaptoethanol at room temperature for 15 min prior to analysis.

tem, 2-amino-2-methyl-l,3-propanediol (AMPD) was used. pH-dependent precipitation occurred already at pH 8.5 and was maximal at pH 9 and 10 in AMPD buffer (Figure 2A). Isoelectric focusing electrophoresis of the patient's serum revealed two specific bands migrating in the range between pI 8.8 and 8.9, which were not present in the normal serum control (Figure 2C). Thus, the apparent pI of the monoclonal protein of 8.8-8.9 was considerably higher than the peak pI of the IgM class of immunoglobulins, which is usually in the range between 5.5 and 6.7 (13). We conclude that the precipitation occurred at minimal net charge of the protein.

Hydrogen bonds or hydrophobic effects seem to be involved in the precipitation of the M-protein, because addition of 8 M urea increased the solubility of the M-protein in AMPD buffer pH 9 to about 90% (Figure 2B). However, the ionic strength of the AMPD buffer was not critical for the precipitation process because the M-protein precipitated similarly when serum was diluted in nine parts of 10 mM, 150 mM or 1 M AMPD buffer pH 9 (data not shown). Pretreatment of serum with 1.7% 2-mercaptoethanol to cleave the disulfide bonds in the IgM pentamer completely prevented precipitation in alkaline pH. Under this condition, a large M-protein of 36 g/L appeared in the β region of the protein profile (Figure 1B). This indicates that disulfide bonds or the presence of pentamers are necessary to initiate precipitation.

The sensitivity of capillary zone electrophoresis for the detection of M-proteins was reported in the range of 95%, and when M-proteins are missed they are typically present in low concentrations (7). However, capillary zone electrophoresis may miss even high concentration M-proteins on rare occasions (5–9). In previous studies, some of these false-negative results could be attributed to an incorrect separation of M-proteins with a high pI or pentamerised IgM. Slight modifications of the buffer systems (5, 6) or addition of 2-mercaptoethanol (8) allowed the detection of some of these M-proteins. Cases of pH-dependent cryoprecipitability (14) and one case of a pH-dependant but temperature-independent precipitation (15) have been described in the literature. However, to our knowledge, this is the first publication showing a pH-dependent precipitation of an M-protein in electrophoresis buffer interfering with its detection by capillary zone electrophoresis. It is possible that also some of the previously published cases of false-negative electrophoresis results (5–9) may be related to pH-dependent precipitation.

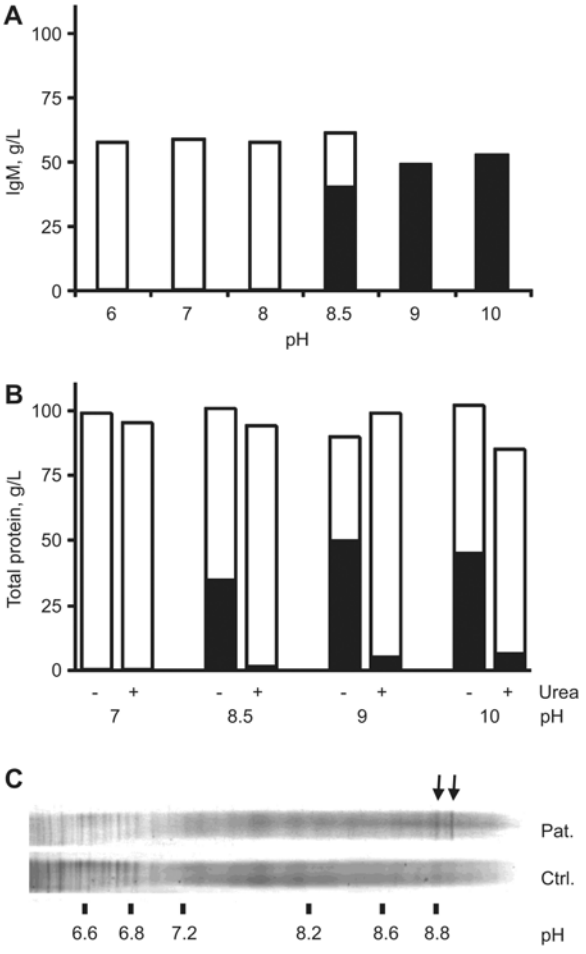

Figure 2 Solubility of the patient's IgMκ depending on pH and urea. (A) Serum with 59 g/L monoclonal IgMκ was adjusted to the indicated pH levels by addition of nine volumes of 0.15 M AMPD buffer. After centrifugation of the precipitates, IgM levels in supernatants (white bars) and precipitates reconstituted with equal volume of AMPD pH 6 (black bars) were determined turbidimetrically on a Modular P800 analyzer (Roche Diagnostics, Switzerland). (B) The same serum was adjusted to the to the indicated pH levels either with 0.15 M AMPD buffer as described above (– urea), or with 0.15 M AMPD buffer containing 8 M urea (+ urea). Precipitates were centrifuged and reconstituted with equal amounts of AMPD pH 6 without or with urea. Total protein was determined in supernatants (white bars) and reconstituted precipitates (black bars) by the biuret method on a Roche Modular P800 analyzer (Roche Diagnostics, Switzerland). Total protein instead of IgM was measured, because urea interfered with IgM quantification. Mean values of two independent experiments are shown. (C) Isoelectric focusing electrophoresis of the patient's serum (Pat.) and of a normal serum control (Ctrl.). Patient's serum diluted 1:1000, normal control serum diluted 1:300, separated on ETC FocusGel 6-11 and stained by silver staining (ETC Elektrophorese-Technik, Kirchentellinsfurt, Germany). Arrows indicate monoclonal bands, the putative monoclonal IgMκ.

When total protein and immunoglobulin concentrations are inconsistent with the native electropherogram, immunofixation and treatment of serum with 2-mercaptoethanol prior to protein electrophoresis are recommended. Further, precipitation of M-proteins at any stage of the analysis has to be considered. Therefore, the serum tube and the dilution segment of the capillary electrophoresis system should be examined for the presence of a precipitate.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.
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