The Importance of Temperature Control in Capillary Electrophoresis of Carbohydrates

キャピラリー電気泳動法による糖鎖解析における、温度制御の重要性

KeyWords：N結合糖鎖解析、Fc融合蛋白、PA800、ファーストグリカンラベリング解析キット、分離特性最適化

医薬品目的モノクローナル抗体及びFc融合蛋白のN-結合糖鎖構造は、作用機序にも関連する重要なCQAの一つです。一方で糖蛋白質ごとに重要な糖鎖をより確実に分離するには、分離特性を修正する必要もあります。
有効長50cmの条件で、キャピラリー温度を変えて分離特性を修正し、etanerceptの糖鎖分離を、近接して検出される３組の糖鎖セットの分離能を通じて考察しています。15、30、45℃での分離プロファイルをFig. 1に示すとともに、A2 – M5、FA2 – A2(6)G1、FA2(3)G1 – A2G2の分離能の変化をFig. 2に示しています。ポリマーを含む泳動液の粘度変化から低温の方が泳動時間は長くなりますが、糖鎖種間の分離はグループによって変わり、双方向の変化が見られました。

これらの考察は、PA800　Plusの正確なキャピラリー温度制御機構に基づいています。さらに全体的に分離を最適化するアイデアとして、キャピラリー温度勾配（泳動中の温度変化）を組み合わせた泳動条件（Fig.　　3）が提案され、etanerceptの糖鎖プロファイルを数値化しています（Table 1）。
医薬品応用の抗体またはFc融合蛋白において、その作用機序からCQAとして重要視される糖鎖種によりフォーカスしての分離が選択できることになります。

The Importance of Temperature Control in Capillary Electrophoresis of Carbohydrates

Introducing Temperature Gradient Capillary Electrophoresis

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Overview

Who Should Read This: Senior Scientists, Lab Directors in Biopharma

Focus: We introduce temperature gradient capillary electrophoresis to enhance the selectivity of complex carbohydrates using the PA 800 Plus Pharmaceutical Analysis System with the Fast Glycan Sample Preparation and Analysis Kit.

Goal: To fine tune the separation selectivities of closely migrating carbohydrate species of high CQA importance.

Problem: The activation energy requirement of carbohydrates during the electromigration process is separation temperature dependent.

Results: Understanding the phenomena of temperature dependent differential migration shifts of branched N-glycan subtypes in capillary electrophoresis allowed fine-tuning of the resolving power between carbohydrate structures of CQA importance. Closely migrating N-glycan structure pairs showed resolution maximums at different temperatures, suggesting the temperature gradient method as an easy and fast way to find out their optimal separation temperature.

Key Challenges:

Separation selectivities of carbohydrates are highly temperature dependent.

Key Features:

The precise temperature control of the PA 800 Plus Pharmaceutical Analysis System provides proper conditions for optimized analysis of complex carbohydrates.

The PA 800 Plus Pharmaceutical Analysis System

Introduction

The general assumption in capillary electrophoresis (CE) is that elevated temperature adversely affects peak resolution, due to the increased diffusion rate of the migrating analyte molecules. However, it has been recently reported that the activation energy requirement of the electromigration process is separation temperature dependent, therefore, the temperature parameter can be utilized in resolution manipulation.[1] Here we discuss the temperature effect on separation selectivities in CE between high mannose and complex type branched N-glycans on a fusion protein of high biotherapeutic interest, etanercept (Enbrel®). PNGase F released glycans were aminopyrene trisulfonate labeled and separated in the temperature range of 15° C to 45° C in 5° C intervals by capillary electrophoresis with laser induced fluorescent detection. It was found that within this temperature range the separation selectivities were highly carbohydrate structure dependent.[2] Structural assignment of the released N-glycans was implemented by utilizing the three internal standard based automated GU value calculation features[3] of the Fast Glycan Labeling and Analysis Kit and the built in database.

Materials and Methods

A PA 800 Plus Pharmaceutical Analysis System was used for all capillary electrophoresis analyses (SCIEX, Brea, CA) equipped with a solid state laser based fluorescent detector (λex=488 nm/ λem=520 nm). The separations were accomplished in 50 cm effective length (50 µm I.D.) bare fused silica capillary columns in the temperature range of 15° C to 45° C at every 5° C with ±0.1° C precision. All runs were done in triplicates. The Fast Glycan Sample Preparation and Analysis kit was used for sample preparation and analysis and the 32Karat, version 10.1 software package for data acquisition (both from Sciex). The etanercept (Enbrel®) was a kind gift of the Medical School of University of Debrecen. All other chemicals were from Sigma Aldrich (St Louis, MO).

Results and Discussion

First, the temperature dependent electrophoretic mobilities of the APTS labeled etanercept glycans were investigated in the range of 15 - 45° C in 5° C intervals. Figure 1 compares the resulting electropherograms at these temperatures, normalized to the lower (DP2) and higher (DP15) bracketing standards as sample window. While the migration times of all oligosaccharides decreased with the increasing temperature, alterations were found in the relative migration time differences between some of the glycans.

Figure 2 delineates the CE-LIF resolution changes between several commercially available glycan standards (which were also found in the Enbrel® N-glycan pool) as a function of the

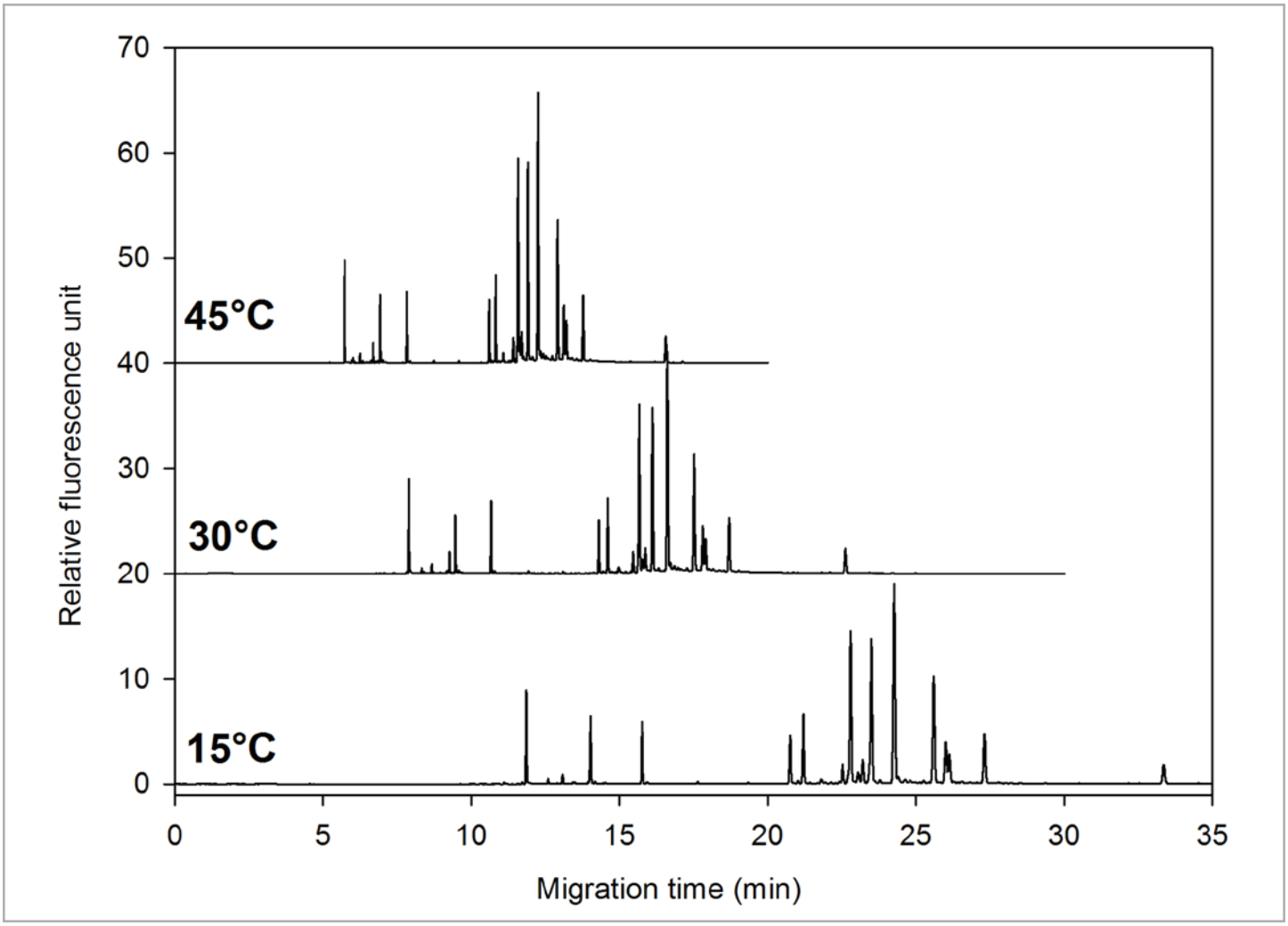

Figure 1. CE-LIF analysis of APTS labeled etanercept (Enbrel®) N-glycans in the temperature range of 15 - 45° C. The sample window is defined by the lower (DP2) and upper (DP15) bracketing standards. Conditions: 50 cm effective length (60 cm total), 50 µm ID bare fused silica capillary, HR-NCHO separation matrix, E = 500V/cm, Injection: pre-injection of water for 5.0 sec at 5.0 psi was followed by 1.0 kV/1.0 sec sample injection and 1.0 kV/1.0 sec bracketing standard.

separation temperature. As one can observe with increasing separation temperature, the resolution between the FA2 and A2(6)G1, as well as the FA2(3)G1 and A2G2 peaks increased while the resolution between A2 and Man5 decreased.

The discovery of this phenomenon suggested that temperature could serve as a powerful separation parameter to fine-tune selectivities and the concomitant resolution between closely migrating carbohydrates in capillary electrophoresis. As a matter of fact, the recently introduced temperature gradient capillary electrophoresis approach proved to be an efficient way to rapidly determine the best separation temperature for the sample components of interest in hand.[2] Figure 3 shows a high-resolution separation of the etanercept (Enbrel®) N-glycans by applying a temperature gradient from 15° C to 55° C during the automated capillary electrophoresis separation process. The upper left inset depicts the associated temperature profile. The separated glycan structures are listed in Table 1.

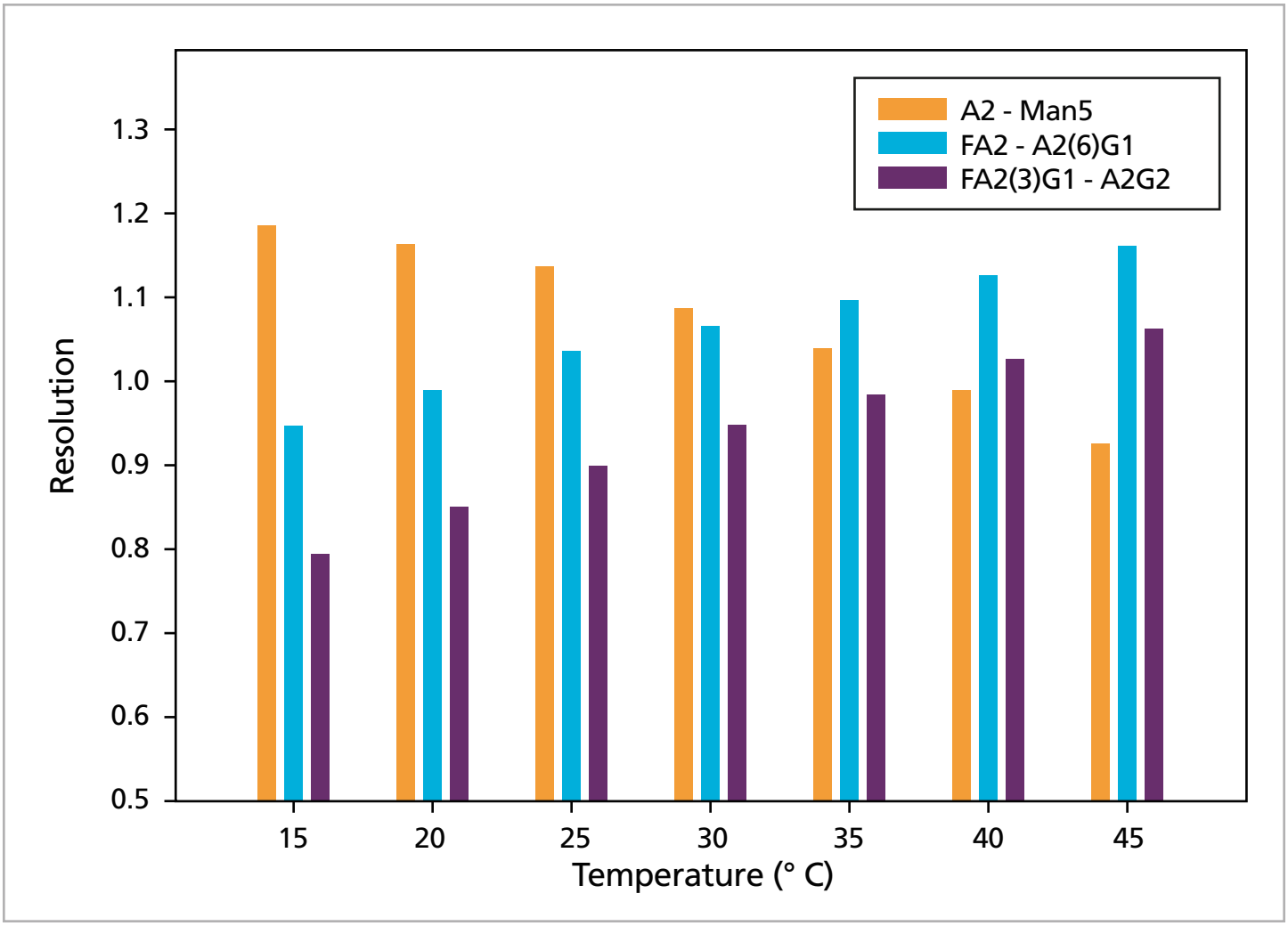

Figure 2. Capillary electrophoresis resolution value changes between the A2/Man5, FA2/A2(6)G1 and FA2(3)G1/A2G2 glycan standards in the temperature interval of 15° C to 45° C.

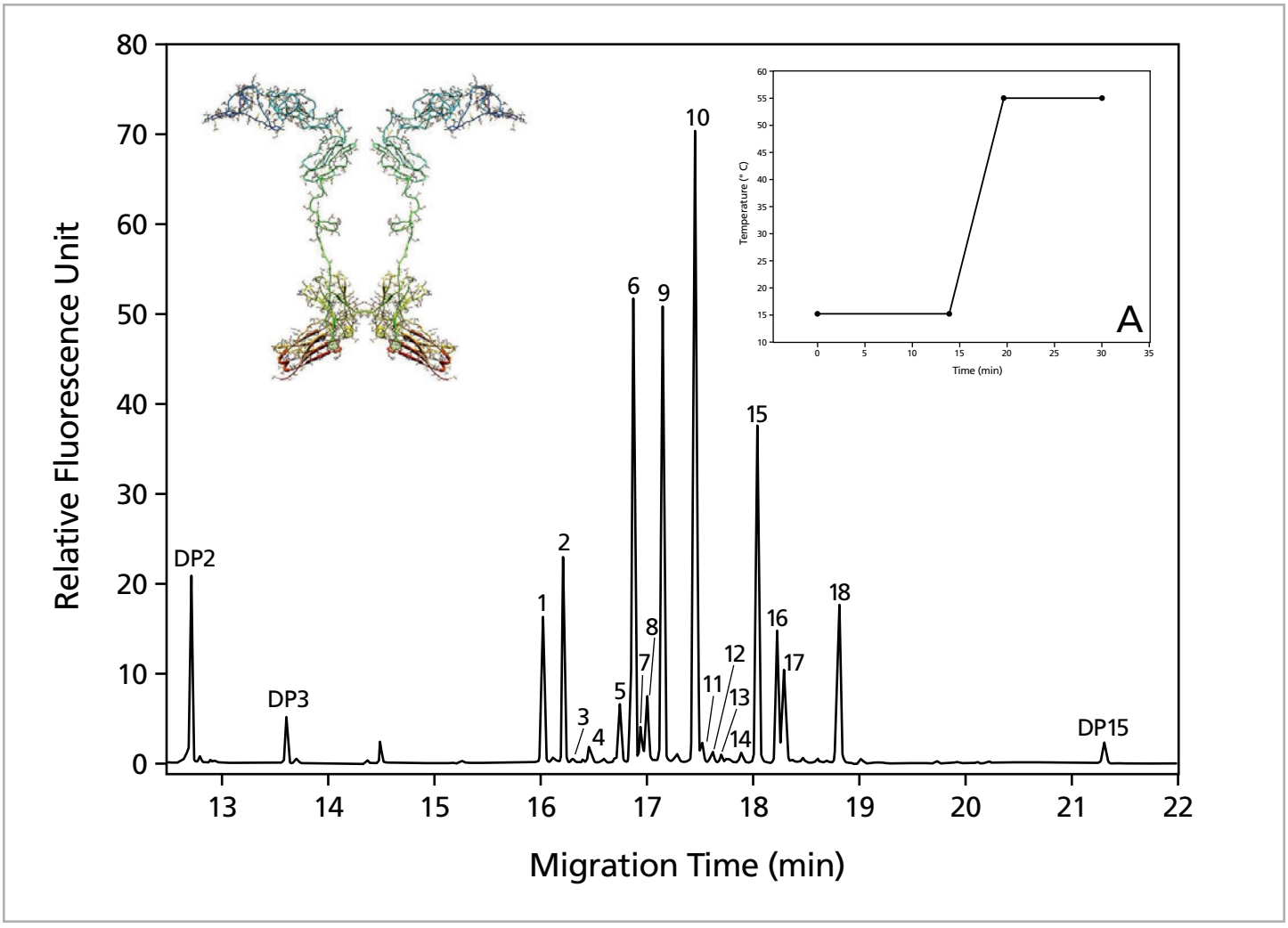

Figure 3. Separation of APTS labeled etanercept (Enbrel®) N-glycans by temperature gradient capillary electrophoresis. Separation conditions were the same as in Figure 1, except the temperature gradient profile that is shown in the inset.

Peak	Peak ID	Migration time (min)	GU Value	Area %
1	A2G2S2	16.05	5.11	3.30
2	FA2G2S2	16.24	5,43	4.04
3	A(6)2G1S1	16.34	5.60	5.74
4	FA(6)2G1S1	16.48	5.84	0.67
5	FA(3)2G1S1	16.78	6.37	1.92
6	A2G2S1	16.90	6.59	13.92
7	A2	16.97	6.72	1.45
8	M5	17.02	6.82	2.27
9	FA2G2S1	17.17	7.09	14.22

Peak	Peak ID	Migration time (min)	GU Value	Area %
10	FA2	17.48	7.67	22.95
11	M6	17.55	7.80	0.73
12	A3	17.64	7.97	0.48
13	A2(3)G1	17.72	8.13	0.31
14	FA3	17.91	8.49	0.40
15	FA2(6)G1	18.06	8.77	12.49
16	FA2(3)G1	18.25	9.11	5.17
17	A2G2	18.32	9.24	3.70
18	FA2G2	18.83	10.19	6.25

Table 1: Identified structures of etanercept (Enbrel®) glycans with the corresponding GU and Area % values.

Conclusions

Understanding the phenomena of temperature dependent differential migration shifts of branched N-glycan subtypes in capillary electrophoresis allowed fine-tuning of the resolving power between carbohydrate structures of CQA importance. Closely migrating N-glycan strucutre pairs showed resolution maximums at different temperatures, suggesting the temperature gradient method as an easy and fast way to find out their optimal separation condition. In the case of etanercept (Enbrel®), the optimum temperature to separate the Mannose 5 oligosaccharide from the closely migrating biantennary non-galactosylated glycan (A2) was 15° C. CE analysis at this temperature provided the necessary information on the important aspect of serum half-life (CQA) expectation of this particular fusion protein therapeutics.

Acknowledgements

The authors gratefully acknowledge the support of Development and Innovation Office (NKFIH) (K 116263) grants of the Hungarian Government.

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Publication number: RUO-MKT-02-6888-B 06/2018

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