Separation of Glutathione and its Novel Analogues and Determination of their Dissociation Constants by Capillary Electrophoresis*

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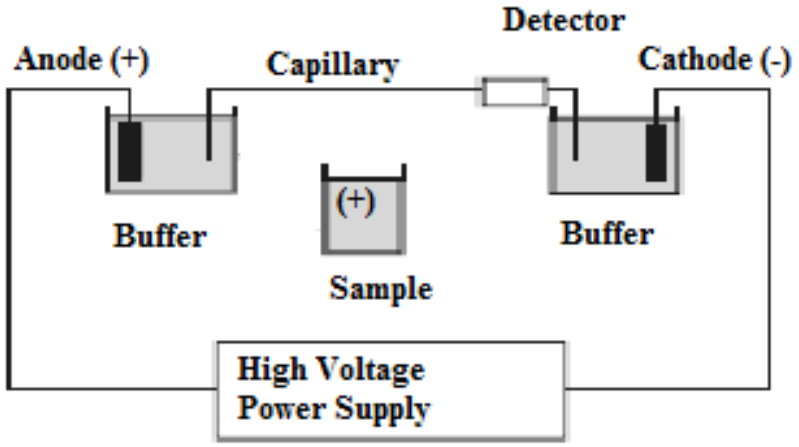
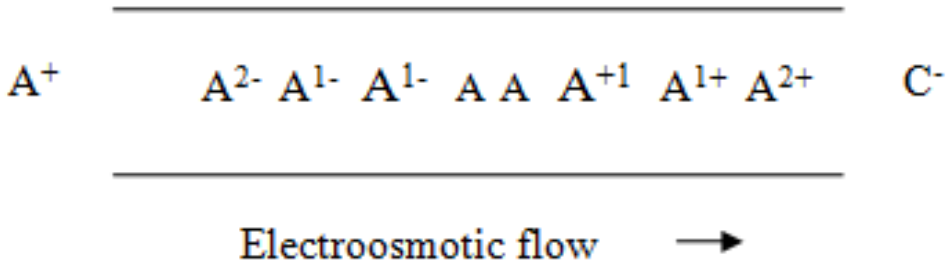
Contents

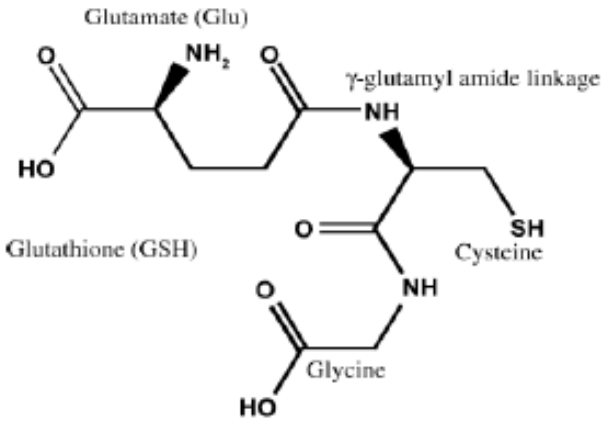
- General
  - Capillary Electrophoresis (CE)
  - Glutathione (GSH) and its Novel Analogues
- Peptide Analysis by CE
  - Separation of GSH and its Novel Analogues &
    - Buffers
    - Peptide mixture identification
  - Determination of their Dissociation Constants
- Conclusions

Capillary Electrophoresis





Glutathione



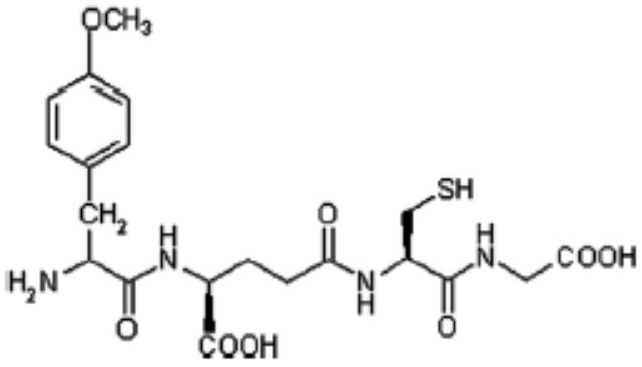


Fig. 1. Structure of UPF1.

- UPF 1 (Tyr(Me)-γ-Glu-Cys-Gly)
- UPF 17 (Tyr(Me)-α-Glu-Cys-Gly)
- MH 33 (Carnosine-Tyr(Me)-γ-Glu-Cys-Gly)
- MH 31 (Carnosine-Tyr(Me)- α-Glu-Cys-Gly
- Carnosine *beta*-alanyl-L-histidine

Peptide Analysis by CE
Separation of GSH and its Novel Analogues

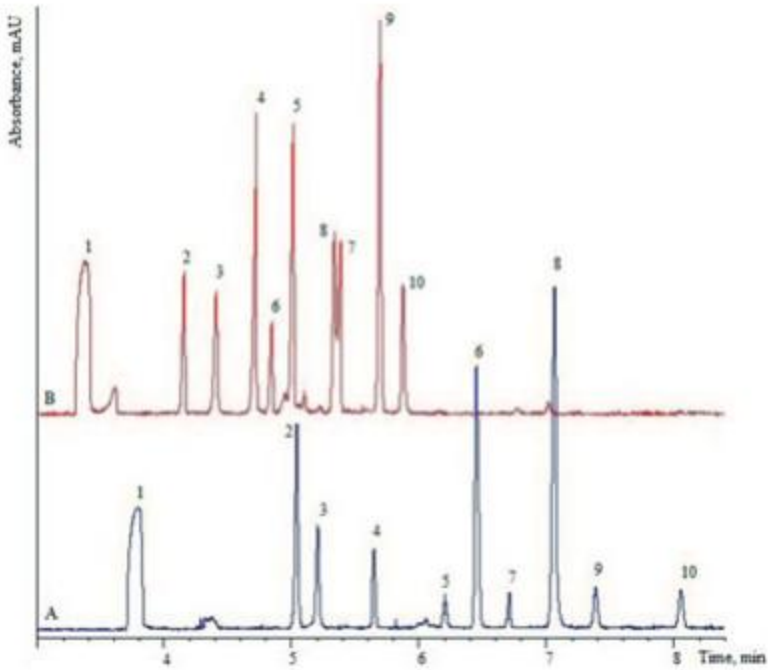


Figure 2. Electropherograms of UPF51, UPF17, and GSH and their homo- and heterodimers at different pH values. CE conditions: 200 mM boric acid as BGE, capillary length 60 cm (51.5 cm to detector), detection at 195 nm, capillary temperature 25°C, injection pressure 50 mbar for 10 s, applied voltage 25 kV. (A) pH 8.45 and (B) pH 7.40. Peak identification: 1—DMF, 2—UPF51, 3—UPF51 homodimer, 4—UPF51 – GSH heterodimer, 5—UPF51 – UPF17 heterodimer, 6—GSH, 7—GSSG, 8—UPF17, 9—UPF17 – GSH heterodimer, 10—UPF17 homodimer.

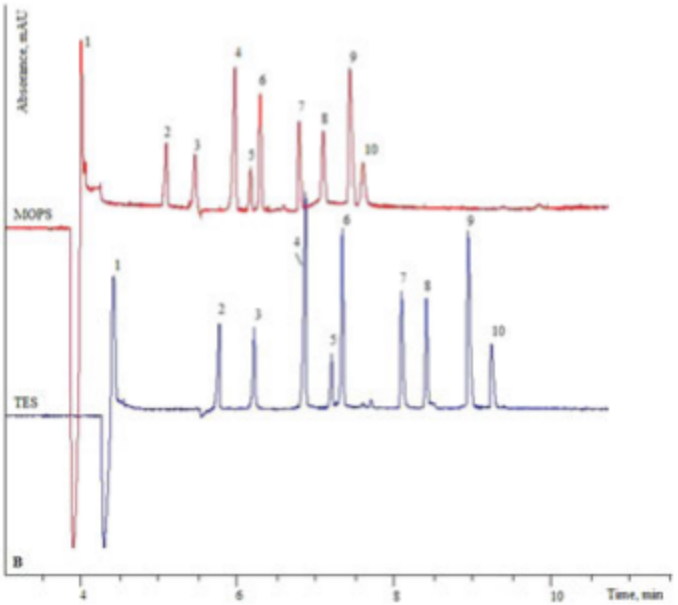


Figure 4. Separation of peptides using CHES/CHES$^-$, TES/TES$^-$, and MOPS/MOPS$^-$ buffers. CE conditions: ionic strength 50 mM, other conditions as in Fig. 2. (A) CHES/CHES$^-$, TES/TES$^-$ buffers at pH 8.45, (B) TES/TES$^-$ and MOPS/MOPS$^-$ buffers at pH 7.80. Peak identification (A): 1—DMF, 2—UPF51, 3—UPF51 homodimer, 4—UPF51 – GSH heterodimer, 5—GSH, 6—UPF51 –UPF17 heterodimer, 7—GSSG, 8—UPF17, 9—UPF17 – GSH heterodimer, 10—UPF17 homodimer. Peak identification (B): 1—DMF, 2—UPF51, 3—UPF51 homodimer, 4—UPF51 – GSH heterodimer, 5—GSH, 6—UPF51 – UPF17 heterodimer, 7—UPF17, 8—GSSG, 9—UPF17 – GSH heterodimer, 10—UPF17 homodimer.

Buffers used

- Inorganic
  - Phosphate buffer (pH 7.40, 7.80, 8.20)- no baseline separation achieved
  - Borate buffer (concentration 50- 250 mM, pH 7.40- 10.00)
- Zwitterionic
  - CHES, TES, MOPS

Determination of pKa-s I

Table 1. Composition of BGEs

pH range covered	Buffer constituents	pK_a
8.45–10.00	$B(OH)_3/B(OH)_4^-$	9.24
7.40–8.20	$H_2PO_4^-/HPO_4^{2-}$	7.21
6.10–5.50	CH_3COOH/CH_3COO^-	4.76
8.45–10.00	$CHES/CHES^-$	9.39
7.80–8.45	TES/TES^-	7.55
7.40–7.80	$MOPS/MOPS^-$	7.18

$$\mu_e = \frac{\mu_{A^-}}{1 + 10^{(pH - pK_a)}}$$

The effective mobility of an ion was calculated as follows:

$$\mu_e = \frac{L_{tot} \cdot L_{eff}}{V}\left(\frac{1}{t_{app}} - \frac{1}{t_{EOF}}\right),$$

where L_{eff} is the distance between the injection point and the detector, L_{tot} is the total capillary length, t_{app} is the migration time of an analyte, t_{EOF} is the migration time of a neutral marker compound, ν_{app} is the apparent velocity, and V is the applied voltage.

Determination of pKa-s II

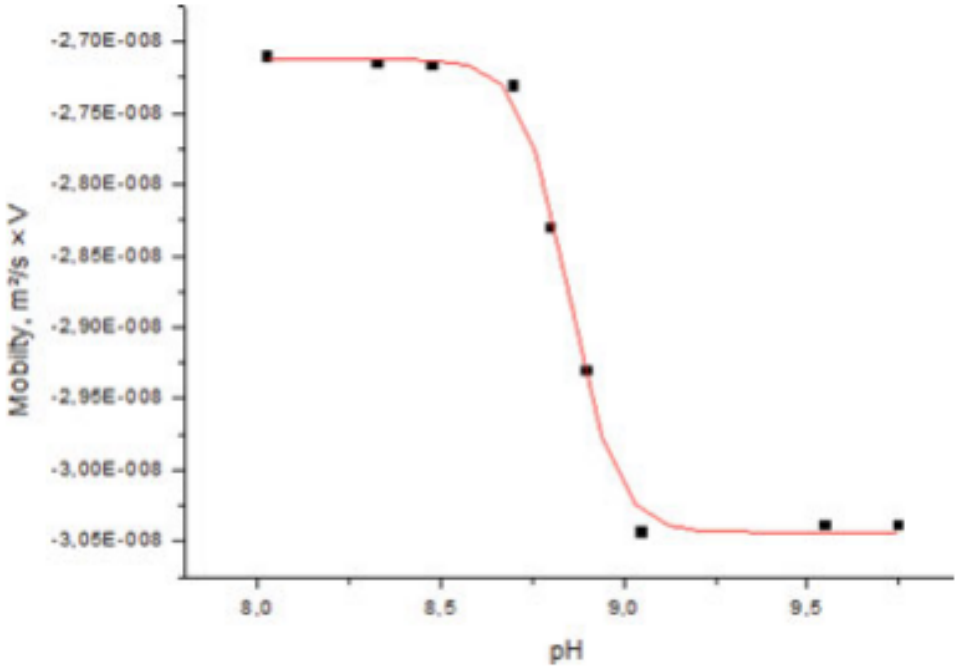


Figure 5. Dependence of the mobility of UPF17 homodimer on pH. Conditions are the same as in Fig. 2, except that 50 mM buffers were used and borate buffer was not used. The inflexion point of the sigmoidal curve corresponds to the pK_a value of the amino group of the UPF17 homodimer.

Determination of pKa-s III

Table 2. Determined pK_a values of some GSH analogues measured by CE ($I = 50$ mM)

Peptide	p$K_a \pm$ SD		
	Imidazolyl	Amino	Thiol
UPF1		8.91 ± 0.05	$8.03 \pm 0.02/9.3 \pm 0.1$[a]
UPF17		8.83 ± 0.07	$7.86 \pm 0.03/9.4 \pm 0.2$[a]
UPF50	6.21 ± 0.05	9.06 ± 0.11	7.89 ± 0.12
UPF51	6.24 ± 0.07	9.00 ± 0.10	7.99 ± 0.10
UPF1 homodimer		9.03 ± 0.08	
UPF17 homodimer		8.84 ± 0.01	
UPF50 homodimer	5.94 ± 0.03	8.95 ± 0.03	
UPF51 homodimer	6.05 ± 0.04	9.01 ± 0.04	
UPF1 – GSH heterodimer		8.90 ± 0.02	
UPF17 – GSH heterodimer		8.96 ± 0.04	
UPF50 – GSH heterodimer	6.19 ± 0.06	8.81 ± 0.04	
UPF51 – GSH heterodimer	6.01 ± 0.02	9.03 ± 0.06	
UPF50 – UPF1 heterodimer	6.20 ± 0.07	9.10 ± 0.07	
UPF51 – UPF17 heterodimer	6.29 ± 0.04	8.93 ± 0.06	
GSH		8.93 ± 0.06	$8.13 \pm 0.4/9.0 \pm 0.3$[a]
GSSG		9.05 ± 0.04	

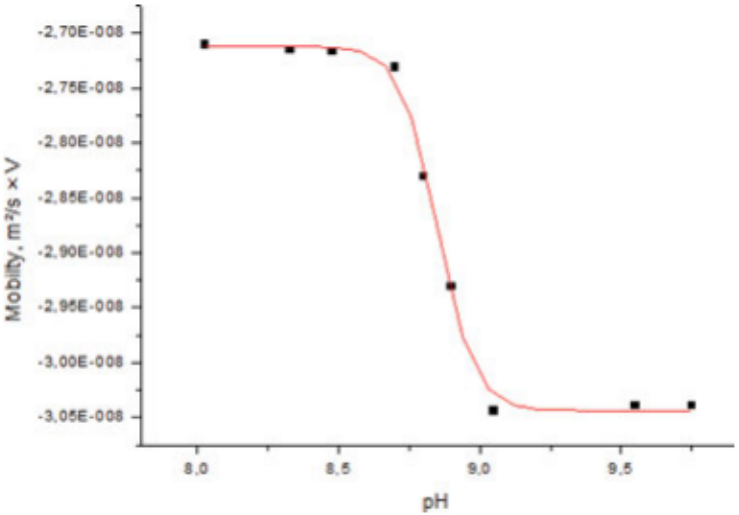
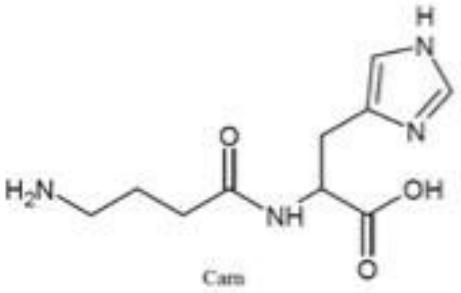


Figure 5. Dependence of the mobility of UPF17 homodimer on pH. Conditions are the same as in Fig. 2, except that 50 mM buffers were used and borate buffer was not used. The inflexion point of the sigmoidal curve corresponds to the pK_a value of the amino group of the UPF17 homodimer.

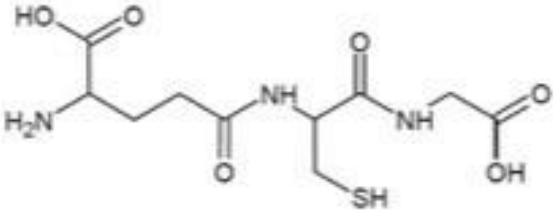
a) Measured pK_a values of thiol groups in GSH, UPF1, and UPF17 by titration [12].

Conclusions

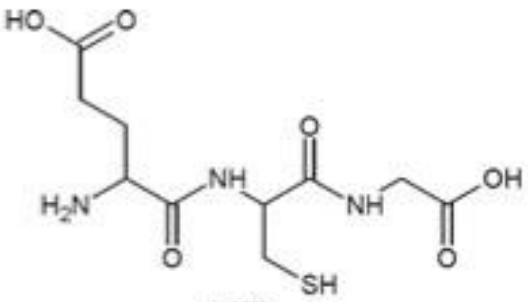
- Separation of GSH and its novel analogues:
  - CE method relatively fast and easy
  - Borate seems to be the best medium for the separation of peptides (high UV transparency compared to CHES, TES, MOPS)
- pKa determination
  - Determination of pKa in a mixture
  - Small amount of sample required
  - Relatively unstable material may be used
  - Impurities do not disturb the measurements



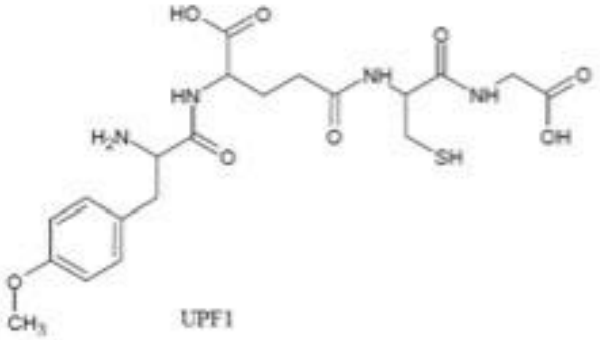
Carn



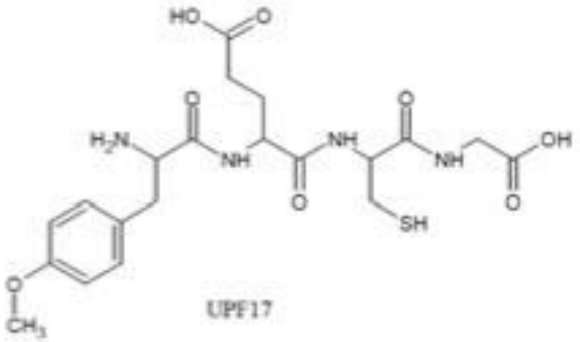
GSH



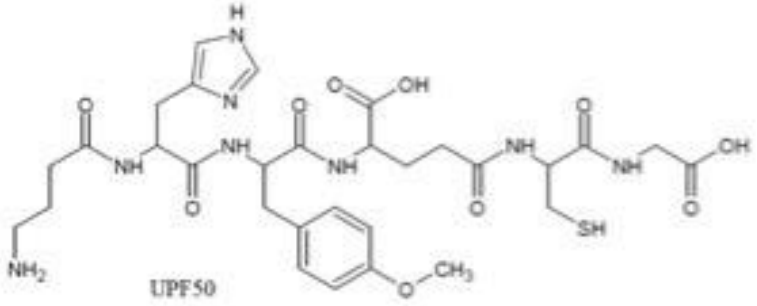
α -GSH



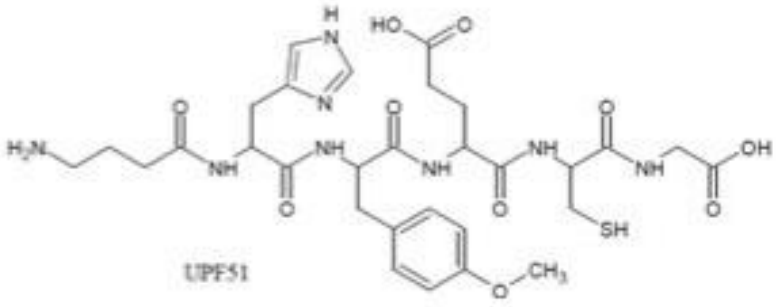
UPF1



UPF17



UPF50



UPF51