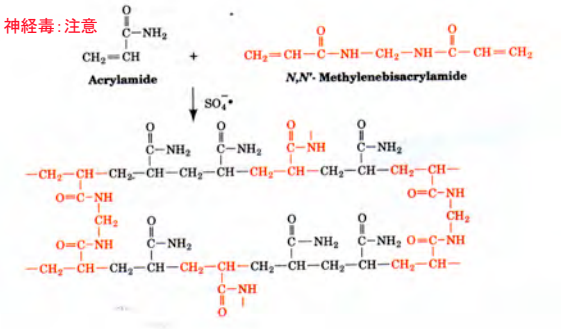
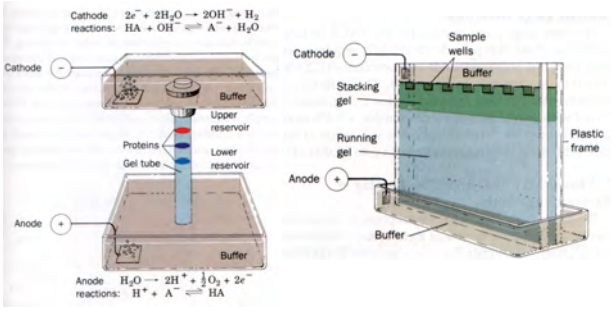
電気泳動の実際 III

神経毒：注意

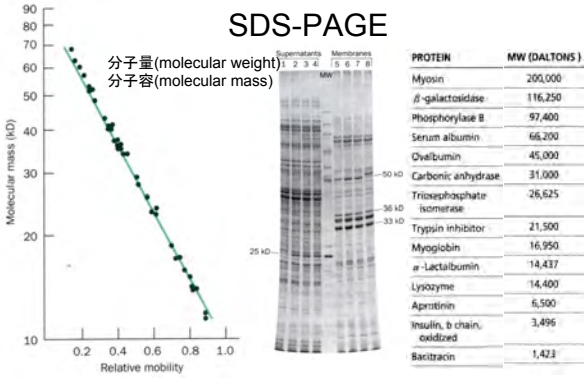


Ammonium persulfate ($S_2O_8^{2-}$ ⇨$2SO_4^{-}$) +N,N,N’,N’-tetramethylethylenediamin によって遊離ラジカルで重合反応開始

電気泳動の実際 IV



SDS-PAGE



PROTEIN	MW (DALTONS)
Myosin	200,000
β-galactosidase	116,250
Phosphorylase B	97,400
Serum albumin	66,200
Ovalbumin	45,000
Carbonic anhydrase	31,000
Triosephosphate isomerase	26,625
Trypsin inhibitor	21,500
Myoglobin	16,950
α-Lactalbumin	14,437
Lysozyme	14,400
Aprotinin	6,500
Insulin, b chain, oxidized	3,496
Bacitracin	1,423

ドデシル硫酸ナトリウム・2MEを加えることで蛋白質を変性させ、分子量に従って分離出来る

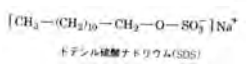
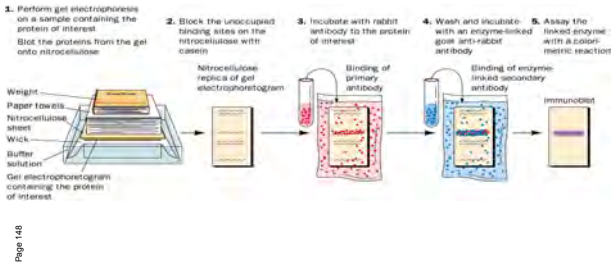


Figure 6-23 Detection of proteins by immunoblotting.



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等電点電気泳動：小分子量（300~600D）のオリゴマーで等電点の連続的に異なるものを作り（キャリアーアンフォライト）、電圧をかける。尿素を加えることが多い。

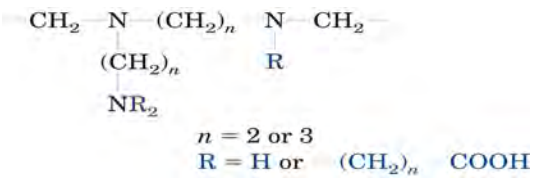


Figure 6-26 General formula of the ampholytes used in isoelectric focusing.

等電点電気泳動：小分子量（300~600D）のオリゴマーで等電点の連続的に異なるものを作り（キャリアーアンフォライト）、電圧をかける。尿素を加えることが多い。

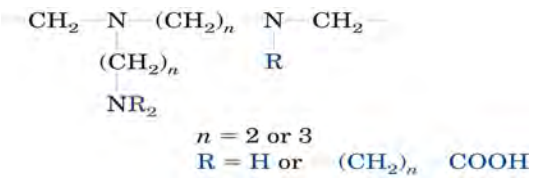
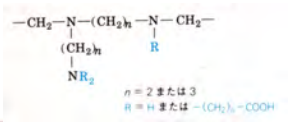


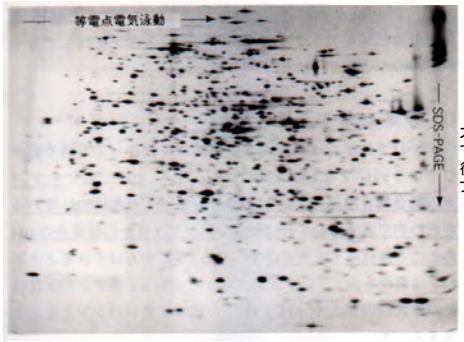
Figure 6-26 General formula of the ampholytes used in isoelectric focusing.

2次元電気泳動

（O'Farrellの電気泳動）

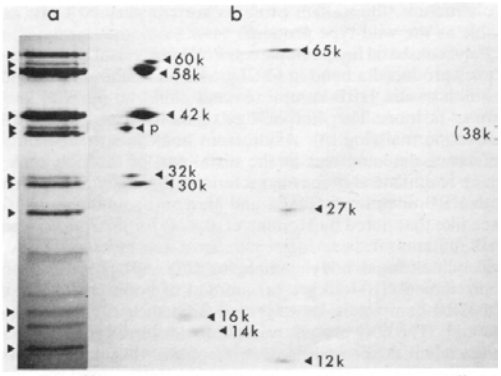


アンホライト
（両性電解質）



大腸菌を[^{14}C]アミノ酸でラベルし、電気泳動後、オートラジオグラフィーで検出

^{35}S-labeled HBB preparation

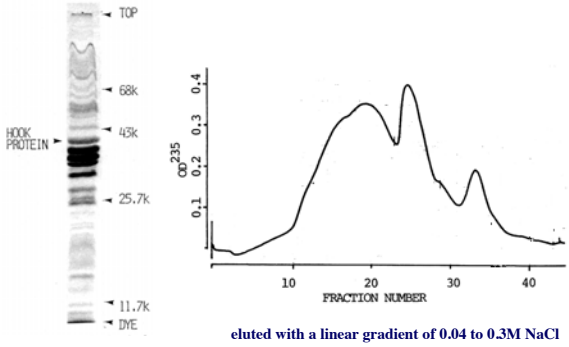


Aizawa et al., J. Bacteriol. (1985)

Protocol for the isolation of hook

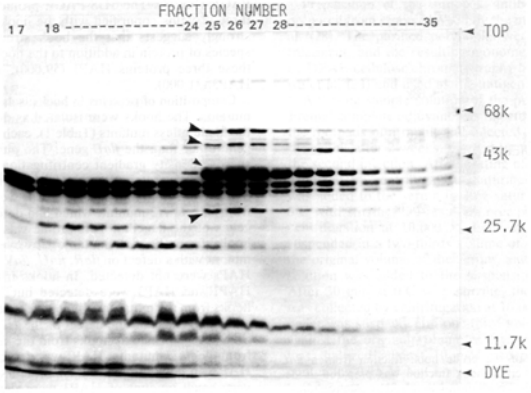
Bacterial pellet (late log phase)
| suspended in 50TN
| homogenizer
| 10,000 x g for 20 min
Sup
| 78,000 x g for 90 min
Ppt
| suspended in 50TNET
| 0°C for 30 min
| 15,000 x g for 15 min
Sup
| 78,000 x g for 90 min
Ppt
| suspended in 10T
| 15,000 x g for 15 min
Sup (crude hook)
| DEAE-cellulose
| 0.04 to 0.3 M NaCl
Hook fraction

Crude hook fraction from a flaL mutant and the DEAE chromatograpy separation of the fraction

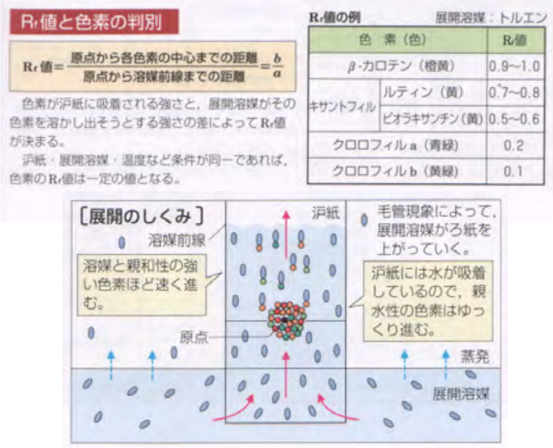


eluted with a linear gradient of 0.04 to 0.3M NaCl

SDS-PAGE of the DEAE fractions from the *flaL* mutant hook

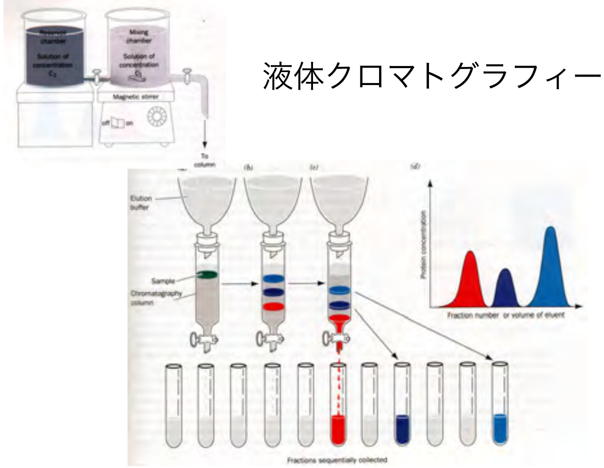


ペーパークロマトグラフィー1



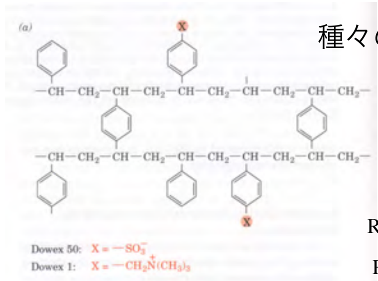
ペーパークロマトグラフィー2

液体クロマトグラフィー



液体クロマトグラフィー AKTAシステム

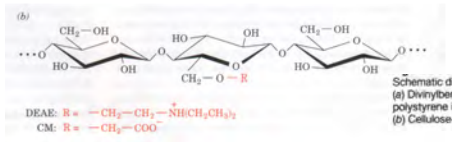
種々のイオン交換体



pHや塩濃度を変化させることで調節

$R^+A^- + B^- \leftrightarrows R^+B^- + A^-$

R^+＝陰イオン交換体



Schematic diagrams of ion exchangers: (a) Divinylbenzene cross-linked polystyrene ion exchange resin. (b) Cellulose-based ion exchangers.

Name[a]	Type	Ionizable group	Remarks
DEAE-cellulose	Weakly basic	Diethylaminoethyl —$CH_2CH_2N(C_2H_5)_2$	Used to separate acidic and neutral proteins
CM-cellulose	Weakly acidic	Carboxymethyl —CH_2COOH	Used to separate basic and neutral proteins
P-cellulose	Strongly and weakly acidic	Phosphate —OPO_3H_2	Dibasic; binds basic proteins strongly
Bio-Rex 70	Weakly acidic, polystyrene-based	Carboxylic acid —COOH	Used to separate basic proteins and amines
DEAE-Sephadex	Weakly basic cross-linked dextran gel	Diethylaminoethyl —$CH_2CH_2N(C_2H_5)_2$	Combined chromatography and gel filtration of acidic and neutral proteins
SP-Sepharose	Strongly acidic cross-linked agarose gel	Methyl sulfonate —CH_2SO_3H	Combined chromatography and gel filtration of basic proteins
CM Bio-Gel A	Weakly acidic cross-linked agarose gel	Carboxymethyl —CH_2COOH	Combined chromatography and gel filtration of basic and neutral proteins

[a]Sephadex and Sepharose gels are manufactured by Amersham Pharmacia Biotech (Piscataway, New Jersey); Bio-Rex resins and Bio-Gel A are manufactured by BioRad Laboratories, Hercules, California.

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Table 6-2 Some Biochemically Useful Ion Exchangers.

ゲル濾過クロマトグラフィー

（分子量の推定や脱塩も行える）

V_X(ビーズ容積)　V_T（ベッド容量）＝ $V_O + V_X$　ブルーデキストラン＝V_Oのマーカー