Recombinant human insulin

III☆. High-performance liquid chromatography and high-performance capillary electrophoresis control in the analysis of step-by-step production of recombinant human insulin

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ABSTRACT

The production of recombinant human insulin consists of five main stages, accompanied by considerable transformation of molecules, concerning size, secondary structure and the presence of charged groups. The application of different methods, *i.e.*, size-exclusion, ion-exchange and reversed-phase high-performance liquid chromatography (HPLC) and high-performance capillary electrophoresis (HPCE) (capillary zone electrophoresis and micellar electrokinetic capillary chromatography), to the analysis of insulin, insulin-related and non-insulin-related substances was studied. A combined HPLC–HPCE system for the step-by-step control of recombinant human insulin production technology is suggested. The advantages and shortcomings of these methods are discussed.

INTRODUCTION

Insulin is an important protein in practical medicine, and it is advisable to carry out its production by microbiological methods. Recom-

binant human insulin (Fig. 1) is formed in the course of fermentative splitting of proinsulin, which is in turn is obtained from fusion protein, isolated from "inclusion bodies" of the producer's ground recombinant cells. Recombinant strains of *Escherichia coli* with transformed plasmid are used as the producer [1,2]. In the given case, we used a strain with a built-in plasmid nucleotide sequence, expressing fusion protein, which consists of linear proinsulin and protein A fragments, linked with the N-terminus by means of a methionine residue. The cultivation of a saturated biomass of recombinant cells ensures the beginning of fusion protein production, the expression and step-by-step transformation of which lead to insulin. Insulin intended for the production of medicaments must be of high

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☆ For Part II, see ref. 27. Abbreviations used: SE-, IO- and RP-HPLC = size-exclusion, ion-exchange and reversed-phase high-performance liquid chromatography; HPCE = high-performance capillary electrophoresis; CZE = capillary zone electrophoresis; MECC = micellar electrokinetic capillary chromatography; rHI = recombinant human insulin; rHP = recombinant human proinsulin; drHP = denatured recombinant human proinsulin; rHP-SSO$_3$ = recombinant human proinsulin-S-sulphonate; rPFP = recombinant proinsulin fusion protein; HMP = high-molecular-mass proteins; TP = theoretical plates.
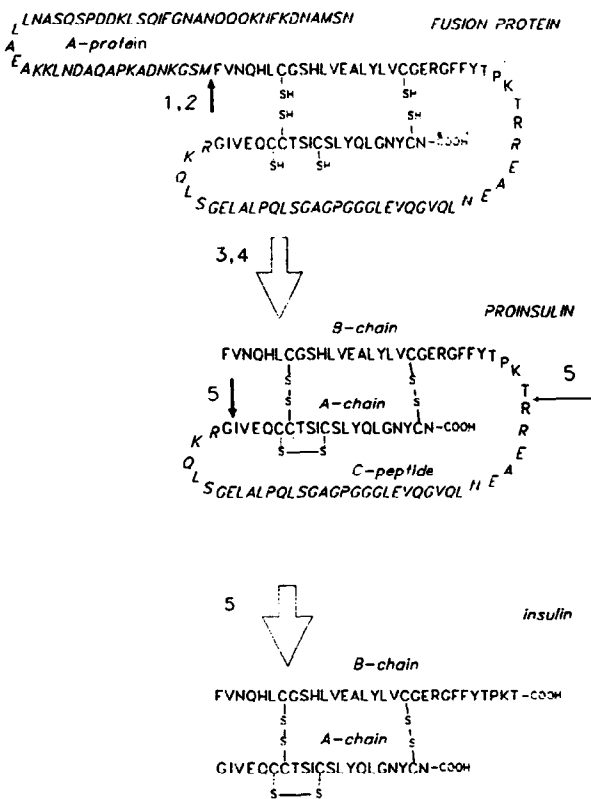
Fig. 1. Transformation of fusion protein molecules into insulin molecules: 1 = rPFP; 2 = rPFP splitting according to methionine residue (M) under the influence of BrCN into linear proinsulin and A-protein fragment; 3,4 = formation of rHP as a result of SH-group sulphitolysis, followed by the closure of S–S bonds in the process of S–SO$_3$ group reduction; 5 = C-peptide restriction out of rHP molecule, accompanied by the formation of rHI.

High-performance capillary electrophoresis (HPCE) is very promising analytical method applicable in many branches of chemistry [16], including biochemistry [17]. The most useful and successfully technique of HPCE for the analysis of biopolymers is capillary zone electrophoresis (CZE) [18]. In spite of problems connected with the adsorption of species on the capillary walls, good results in the analysis of peptides and proteins by CZE have been achieved [19,20]. CZE is considered to be promising for the analysis of recombinant proteins in biotechnology [20–24], where its advantages such as economy [21] and high resolving power [22] are especially important.

EXPERIMENTAL

For SE-HPLC the columns used were TSK G 3000 SW (300 × 7.5 mm I.D.) (TOSOH) and Protein Pack 60 (30 × 0.75 mm I.D.) (Waters–Millipore); elution was performed at a flow-rate of 0.5 ml/min.

Chromatography was carried out using a Varian 8500 pump with a Waters U6K injector, DuPont Model 852001-902 spectrophotometer and Waters Model 740 integrator. For IE-HPLC the columns used were Armsorb-Si-500 poly-Amin (150 × 4 mm I.D.). Armsorb-Si-500 DEAE (150 × 4 mm I.D.), (Armchrom, Yerevan, Armenia), Protein Pac DEAE 5 PW (250 × 4.6 mm I.D.) (Waters,) and Nucleogen DEAE 4000-7 (150 × 4 mm I.D.) (Machery–Nagel), For RP-HPLC the columns used were Armsorb-Si-300 C$_8$ P(DM) (150 × 4 mm I.D.), Armsorb-Si-300 C$_8$ RP-PR (300 × 4 mm I.D.), (ErONEM Archrom), Nucleosil 300-7 Protein RP (150 × 4 mm I.D.), Nucleosil C$_{18}$ (150 × 4 mm I.D.) (Machery–Nagel) and μBondaPak C$_{18}$ (300 × 3.9 mm I.D.) (Waters), Chromatography was carried out using a Waters Model 510 pump with a Waters U6K injector, a Waters Model 490E spectrophotometer and a Waters Model 740 integrator.

For the separation we used specimens of insulin, proinsulin, denatured proinsulin, proinsulin-S-sulphonte, fusion protein (obtained from IBC RAS) and for identification a human insulin standard specimen (Atlanta, Cat. No. 83/500,

purity and contain, for example, not more than 0.1% of proinsulin, not more than 1% of high-molecular-mass proteins, including insulin oligomers, and not more than 1% of desamido-(A$_{21}$)-insulin [3,4]. The separation of insulin and peptides closely related to it is a difficult task because of the small difference in their conformation and charge [3,5]. The use of RP-HPLC for the fine purification of recombinant, human, bovine, porcine rat and other insulin and insulin-related proteins on well known commercial columns has been suggested earlier [3,5–13]. Proinsulin-S-sulphonate (see below), proinsulin, separate A- and B-chains and their S-sulphonates have also been characterized with the help of RP- and IE-HPLC [14,15].

Chemie- und Handelsgesellschaft, Heidelberg, Germany). The reagents used were: acetonitrile, methanol, sodium hydroxide, sodium chloride, sodium sulphate, sodium phosphate, phosphoric acid, acetic acid (all of the highest purity available), water purified on a Milli-Q system (Millipore), sodium dodecyl sulphate (Serva) and guanidine hydrochloride (Merck). Before chromatography, the eluents were filtered through nitrocellulose and GVWP filters (pore diameter 0.45 μm; Millipore) and degassed for 20 min.

For HPCE we used a PACE 2010 system, with fused-silica capillaries of I.D. 100 μm, length 87 cm, effective length 80 cm, and I.D. 100 μm, length 52 cm, effective length 40 cm (Beckman).

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in vertical polyacrylamide gel (thickness 0.7 mm, T = 15%) according to Laemmli [25], at a constant voltage of 220 V. Mass spectrometric analysis was carried out on an MSBX-252Cf instrument. Amino acid analyses were carried out using well known methods [26].

RESULTS AND DISCUSSION

Total scheme of protein transformation

The production of recombinant human insulin includes five main steps, in the course of which considerable transformations of the molecule take place (Table I and Fig. 1). These changes were analysed by SE-, RP- and IE-HPLC and HPCE (CZE and MECC), the process being accompanied by a study of applicability and informativity of chromatographic and electrophoretic methods.

rPFP, produced by recombinant cells and expressed in the first step, which is human proinsulin with open and chaotically closed six cysteine SH groups [2], specifically splits according to the methionine residue under the influences of BrCN into linear ("denatured") proinsulin and A-protein fragment (stage 2).

As a result of the denatured proinsulin sulphitolysis reaction, cysteine SH groups are converted into SSO_3 groups of proinsulin-S-sulphonate (stage 3). Subsequently, proinsulin-S-sulphonate is reduced and renatured in the presence of β-mercaptoethanol (stage 4); the "curling up" of the proinsulin molecule on the closure of the S–S bridges takes place. The final stage 5, under the influence of trypin, is the formation of insulin, the structure and properties of which do not differ from those of human hormone [2,4].

The authenticity of insulin obtained according to the given method at the Institute of Bioorganic Chemistry of the Russian Academy of Sciences was corroborated by HPLC with a standard specimen, SDS-PAGE (insulin appears in the form of a single stripe, corresponding to M_r 5800 ± 500), analysis of the N-terminal amino acid sequence and complete amino acid composition, and mass spectrometry (the insulin molecular mass was found to be 5808.2). The physiological activity of the protein proved to satisfy modern requirements, i.e., more than 27 u/mg [4].

As purity of intermediate products is necessary for the correct performance of the technological steps, this work was devoted to developing the control system for the main stage of recombinant human insulin production with the help of different highly effective chromatographic and electrophoretic methods. The necessity to use separate types of HPLC and HPCE and their combinations for the complete analysis of technological products in each step has been demonstrated. The use of specially developed (in cooperation with Armchrom) laboratory-made Armsorb HPLC columns for proteins was studied. Parameters for the column support were obtained and conditions for carrying out chromatography were established such that the resolution and selectivity in the separation of protein products were comparable to those obtained on foreign commercial analogues.

HPLC analysis

It is necessary to use size-exclusion HPLC along with reversed-phase and ion-exchange chromatography because in the process of transformation of the initial FP into insulin considerable change in the size and structure of the molecules take place (Table I). In using known methods and developing new ones, we were limited by the conditions of preventing oxidation and denaturation of protein molecules and the

TABLE I

MAIN STEPS AND INTERMEDIATE AND FINAL PRODUCTS OF RECOMBINANT HUMAN INSULIN PRODUCTION TECHNOLOGY

Steps No.	Step	Step products[a]	Molecular mass ($\times 10^{-3}$)	Using of HPLC and HPCE methods for product analysis				
				IE	RP	SE	CZE	MECC
1	Fusion protein isolation	*rPFP*	17.0			+	+	
		Dimeric rPFP	34.0					
		HMP	40–70					
2	Proinsulin denaturation	*Denatured proinsulin*	9.0			+		
		rPFP	17.0					
		HMWP	70.0					
3	Proinsulin sulphiting	*Proinsulin-S-sulphonate*	9.5		+		+	
		Incompletely sulphited proinsulin	9.0					
		rPFP	17.0					
		Fusion protein-S-sulphonate	17.5					
4	Proinsulin renaturation	*Proinsulin*	9			+	+	
		Structural analogues and oligomers	9 / 18–36					
5	Insulin production	*Insulin*	5.8		+	+	+	+
		Insulin-like proteins	5.7					
		Deamidated insulin	5.75					
		Proinsulin	9					
		HMWP	6–36					

[a] Main products in italics.

corrosive resistance of the instruments. Conditions were found under which all the main products of insulin production technology are separated (Fig. 2). It should be noted that, although proinsulin, proinsulin-S-sulphonate and denatured proinsulin (*i.e.*, open proinsulin with free SH groups of cysteine residues) have close molecular masses, they differ considerably in conformation and charge [14,15]. It is these differences that explain the possibilities for their separation, and although the mechanism of separation is not purely size exclusion in character, a sufficiently high resolution between protein peaks was achieved (Fig. 2). Detailed information about the SE-HPLC of insulin-containing products can be found elsewhere [27].

In the first step, when the FP is expressed, the content of the main product with reference to oligomers and high-molecular-mass proteins (*E. coli* metabolites) is analysed by the given size-exclusion system (Table I), and rPFP elution on the RP-IE columns is characterized by low
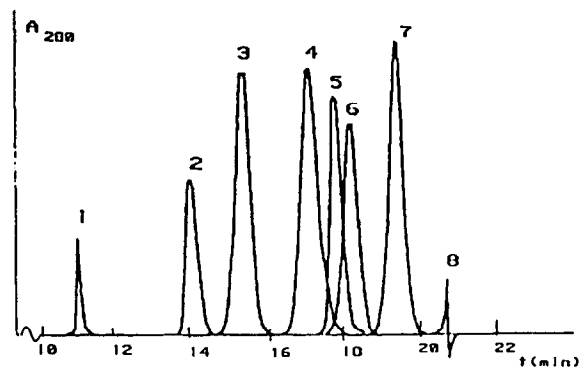
Fig. 2. Superposed chromatograms of insulin-containing proteins, performed on a TSK G 2000 SW column (600 × 8 mm I.D.) with 0.1 M sodium phosphate–0.2 M Na_2SO_4–5% acetonitrile (pH 7.0), flow-rate 1 ml/min. In the analysis of technological products the following proteins, corresponding to every stage in Table I, are present: 1 = HMP; 2 = rPFP dimer; 3 = rPFP; 4 = rHP-SSO_3; 5 = drHP; 6 = rHP; 7 = rHI; 8 = salts.

selectivity of separation and partial protein sorption on the columns. The protein appears in the form of a broad peak and it is difficult to detect the presence of impurities. When analysing the products of BrCN splitting of rPFP (into linear proinsulin and A-protein fragment), we obtain information concerning the completeness of the reaction and the amount of high-molecular-mass impurities in the reaction mixture. The use of RP and IE chromatography in the step of rPFP splitting is also not effective, because, first, these proteins do not process a considerable difference in charge and, second, although linear proinsulin is eluted in the form of separate narrow peak on an RP column, its comparison with non-splitting rPFP is difficult. In this step, SE chromatography is most effective. In the proinsulin sulphitolysis reaction, we found the reaction to be complete made and found in the reaction mixture high-molecular-mass impurities, which are products of protein oligomerization and which are not easily separated from monomers in IE-HPLC. After renaturation of proinsulin and in the final step of insulin production, an analysis is made in order to determine to content of high-molecular-mass impurities, the level of which should not exceed 0.1%, and which are not detected by RP-HPLC owing to the coincidence of their retention times with those of related proteins. It should be noted

that the dynamics of performing the reactions can also be analysed with the help of the given size-exclusion system.

A more effective separation of insulin, proinsulin and proinsulin-S-sulphonate from the accompanying impurities is ensured by RP- and IE-HPLC, carried out on commercial and domestic columns. Anion-exchange HPLC was used for the analysis of proinsulin-S-sulphonate. The presence of SSO_3 groups in this protein leads to its affinity to the anion-exchange support. Fig. 3 shows the separation of proinsulin-S-sulphonate from incompletely sulphonated proinsulin in the form of separate peaks. The sorbent Armsorb-Si-500 poly-Amin is a wide-pore silica (pore diameter 500 Å), modified by a polymer-inoculated phase, carrying amino groups. Columns with this support are characterized by high effectiveness ($N = 7000$ TP per
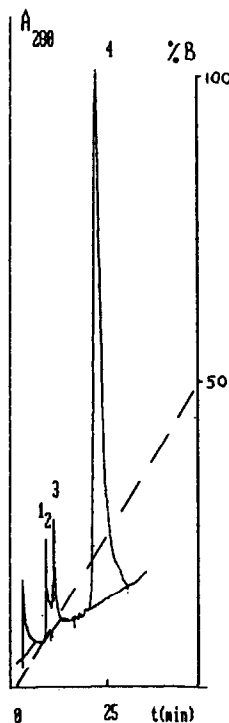

Fig. 3. Chromatogram of reaction mixture of rHP-SSO_3 production (4). Peaks 1–3 represent incompletely sulphonated proinsulin. Column, Armsorb-Si-500 poly-Amin; eluents, (A) 0.02 M sodium phosphate–MeOH (90:10) (pH 7.5); (B) 1 M sodium phosphate–MeOH (90:10) (pH 6.2); flow-rate, 1 ml/min. The percentage of eluent B in the mobile phase is shown by the dashed line.

column for insulin), resolution between peaks (in the given case $R_{s3,4} = 2$) and selectivity ($\alpha_{3,4} = 1.5$) (Fig. 3). In addition, Armsorb-SI-500 poly-Amin is distinguished by stability, chemical resistance and a sufficiently high loading ability.

The resolution between peaks did not change substantially when proinsulin-S-sulphonate was applied to the column in amounts of 2 μg–2 mg. The dependence of the peak area on the amount of sample applied within the specified limits turned out to be linear, which also testified to the high loading ability and high quality of the sorbent surface. After carrying out 1500 analyses (during a year), the resolution and loading ability were same as with a new column. The column was used in the pH range 2.5–8.0 with different organic solvents and salts, with did not affect its physico-chemical properties. The separation of proinsulin-S-sulphonate and incompletely sulphonated proinsulin, carried out on Armsorb-Si-500 poly-Amin columns, was similar to that on commercial columns as far as resolution and selectivity are concerned.

On renaturation (reduction of SSO_3 groups and closure of S–S bridges), along with the formation of proinsulin, the formation of its linear molecular analogues (in the case of incorrect closure of S–S bonds) and oligomers in intramolecular S–S binding is possible. It has been shown above that the determination of oligomers is carried out by means of SE-HPLC, and the analysis of proinsulin proper and the presence of analogues by means of RP-HPLC, selective to these variations in the structure of protein molecules (see Fig. 4 and ref. 3). The separation of proinsulin and insulin and their close analogues is caused by the difference in the hydrophobic properties of these proteins and was carried out on reversed-phase columns. Among several modifications of column supports, the best results were achieved on an Armsorb-Si-300-C_8 P(DM) column (Fig. 4) (wide-pore silica, modified by γ-glycidyl groups with inoculated C_8 phase). Several eluent systems used for the chromatography of proteins and peptides were tested and high resolution was achieved with systems such as acetonitrile–water with TFA, acetonitrile–water with sodium phosphate, acetonirtile–water with NH_4OAc and methanol–
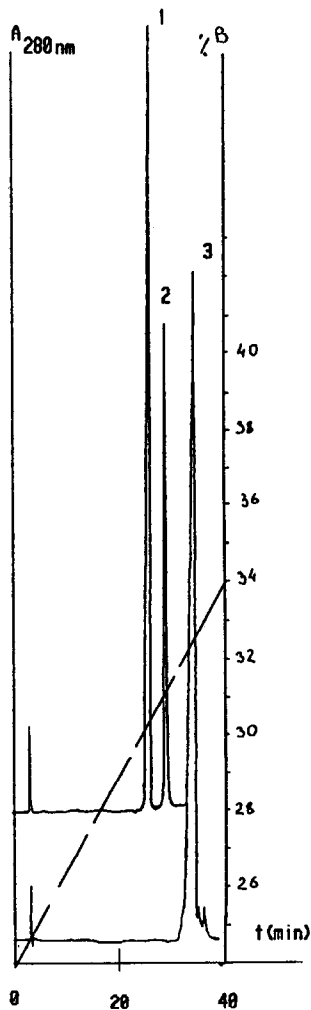

Fig. 4. Analysis of isolated fractions: rHP (peak 3); insulin (peak 1). Peak 2 = desamidoinsulin. Column, Armsorb-Si-300 p (DM); eluents, (A) CH_3CN–1 M NH_4OAc (10:90) (pH 7); (B) CH_3CN–1 M NH_4OAc (50:50) (pH 7); flow-rate, 0.8 ml/min. The percentage of eluent B in the mobile phase is shown by the dashed line.

water with NH_4OAc. The separation was accompanied by good resolution of the insulin, desamidoinsulin and proinsulin peaks ($R_{s1,2} = 2,3$; $R_{s1,3} = 3.8$), owing to the high selectivity, which is especially important in the final steps of purification in the process of the identification of small amounts of these impurities (Fig. 4).

For the analysis of insulin medicaments, it is necessary that the column effectiveness should not be less than 4000 TP/m and the resolution

between insulin and desamidoinsulin not less than 1.8 [4]. In the given case the column effectiveness is 7000 TP per column of length 15 cm. The resolution between peaks (insulin–desamidoinsulin and insulin–proinsulin) is similar to that with commercial columns, and satisfies the necessary requirements (Table II) [4]. The columns were tested for loading ability, stability and chemical resistance with the help of the above-mentioned method with similar results.

HPCE analysis

In order to determine the advisability of including HPCE in a set of techniques using during the process of production of recombinant human insulin (rHI), we carried out investigations of its applicability in all stages of this process. The main technique used was CZE, but some interesting results was obtained with MECC.

Analysis of recombinant human proinsulin fusion protein (rPFP) by CZE gave interesting results. As mentioned above, the same analysis by SE-HPLC demonstrated three peaks (Table I, Fig. 2). We identified first the monomer and dimer of rPFP and other oligomers generated by chaotic formation of disulfide bonds, which were not separated by SE-HPLC because they have similar dimensions. However, CZE demonstrated many peaks (Fig. 5a). Probably this phenomenon can be explained from the position of charge heterogenity of the oligomers with different numbers of disulphide bonds and different conformations.

It is obvious that the presence of oligomers hinders any analysis. In order to achieve monomerization we treated a sample of rPFP with 2-mercaptoethanol (ME). The fact that monomerization carried by this procedure out is exhaustive was proved by CZE of chromatographically refined renatured rPFP (rPFP) monomerized by ME (Fig. 5b). Monomerization of rPFP allowed us to obtain good results in the analysis of mixture of rPFP and contaminated proteins of host cells (Fig. 5c). ME gave a separate peak because it was injected with the sample, but addition of ME to the buffer solution is impossible owing to its high UV adsorption at the wavelength used. The same approach was demonstrated by Patrick and Lagu [28] in the analysis of rPFP by SE-HPLC.

MECC separation of rPFP monomerized by ME resulted in wide peak broadening, which excluded the possibility of any analysis.

We consider that rPFP, which is able to form many isomers differing in many, charge and conformation, is a useful model for studying of the laws and driving forces of CZE and MECC separations.

Insulin-containing proteins devoid of free cysteine groups are readily separated by both CZE (Fig. 6a) and MECC (Fig. 6b). Recombinant human proinsulin (rHP) was in all instances

TABLE II

COMPARISON OF RESOLUTIONS (R_s) OF DIFFERENT SERIAL COMMERCIAL (NOS. 1–3) AND DEVELOPED (ARMSORB) RP COLUMNS FOR THE SEPARATION OF INSULIN, DESAMIDO-(A_{21})-INSULIN AND PROINSULIN

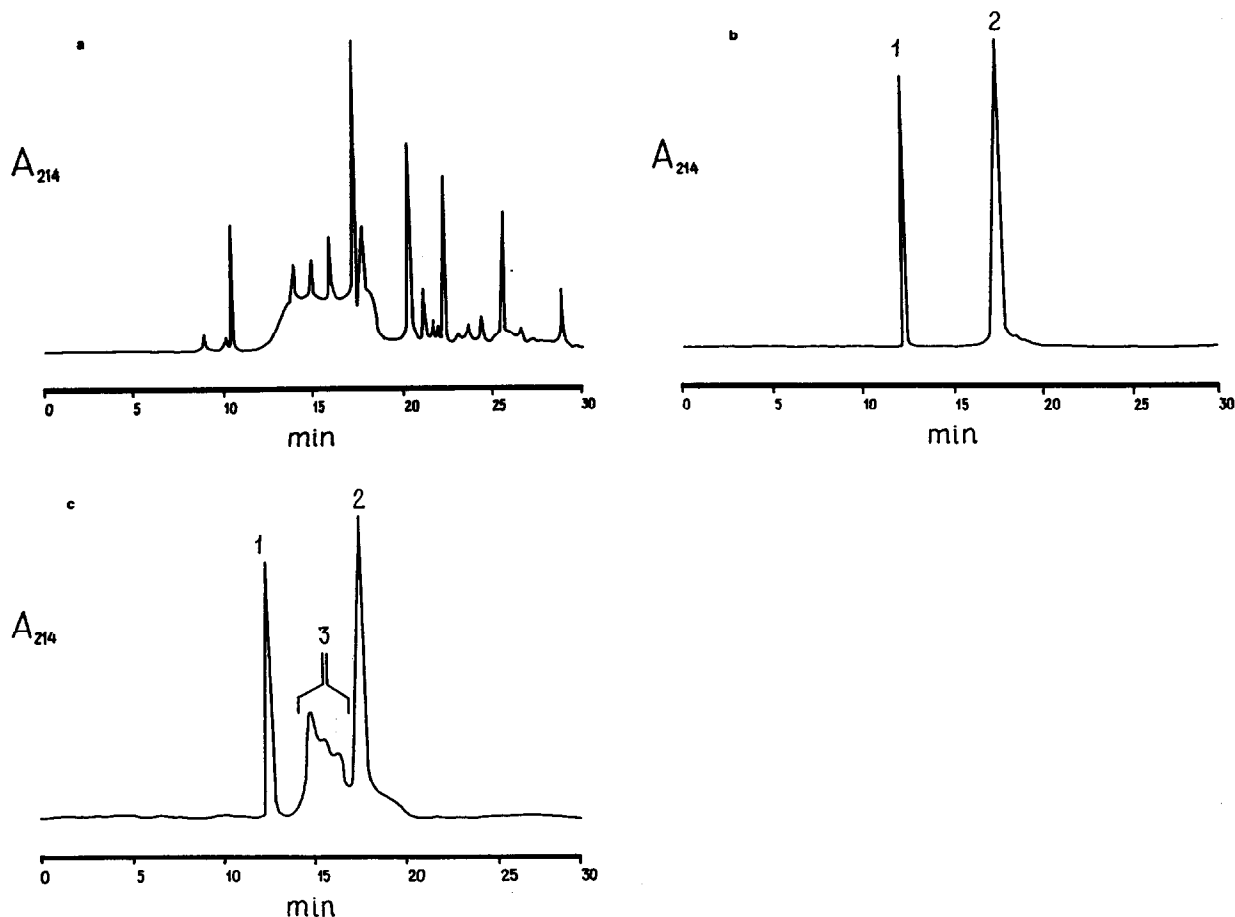| Column	R_s	
	Insulin–desamidoinsulin	Insulin–proinsulin
No. 1: C_{18} (250 × 4.6 mm I.D.)	0.4–1.11	3.0–4.85
No. 2: C_{18} (300 × 3.9 mm I.D.)	1.8	–
No. 3: C_{18} (250 × 4.6 mm I.D.)	1.8	–
Armsorb-Si-300-C_8-RP-PR (150 × 4 mm I.D.)	2.3	6.5
Armsorb-Si-300-C_8-(P)DM (150 × 4 mm I.D.)	2.2	6.3

Fig. 5. CZE of recombinant human proinsulin fusion protein (rPFP). (a) Separation of oligomers of rPFP; (b) analysis of pure renatured rPFP (rPFP-r) treated with 2-mercaptoethanol (ME). ME was added to the sample tube in stoichiometric amounts and mixed. Analysis was performed immediately without further treatment. Peaks were identified by migration times of standards: 1 = ME; 2 = rPFP. (c) Analysis of rPFP in mixture with contaminating proteins of host cell. Peak 3 = contaminating proteins. Analysis was performed using the Beckman PACE 2010 system. Conditions: fused-silica capillary supplied by Beckman, I.D. 100 μm, length 87 cm, effective length 80 cm; voltage, 10 kV; temperature, 28°C; buffer, 0.1 M borate (pH 9.3); concentration of protein in sample, 1 mg/ml; electrophoretic injection, 5 s/+5 kV; detection, UV absorbance at 214 nm.

satisfactorily separated from recombinant human insulin (rHI). rPFP was badly separated from rHP by CZE but satisfactorily by MECC.

During the investigations we typically met in CZE the problem of protein adsorption on the capillary walls. Optimization of the separation conditions allowed us to minimize the adsorption and to achieve an efficiency of 200 000 TP and a selectivity $\alpha = 1.8$ for the separation of rHP and RHI by CZE (Fig. 7a). This results are an order of magnitude higher for this pair them with RP-HPLC.

In spite of the impossibility of protein adsorption during analysis by MECC, the efficiency of separtion of rHP and rHI by this technique was lower than that using CZE. The causes of this phenomenon and methods for optimizing MECC separations of proteins are under investigation.

An important application of the CZE analysis of rHI is in the certification of pharmaceutical insulin according to pharmacopoeial requirements. Other groups have worked in this area [20–23]. Nielsen et al., [23], in a significant
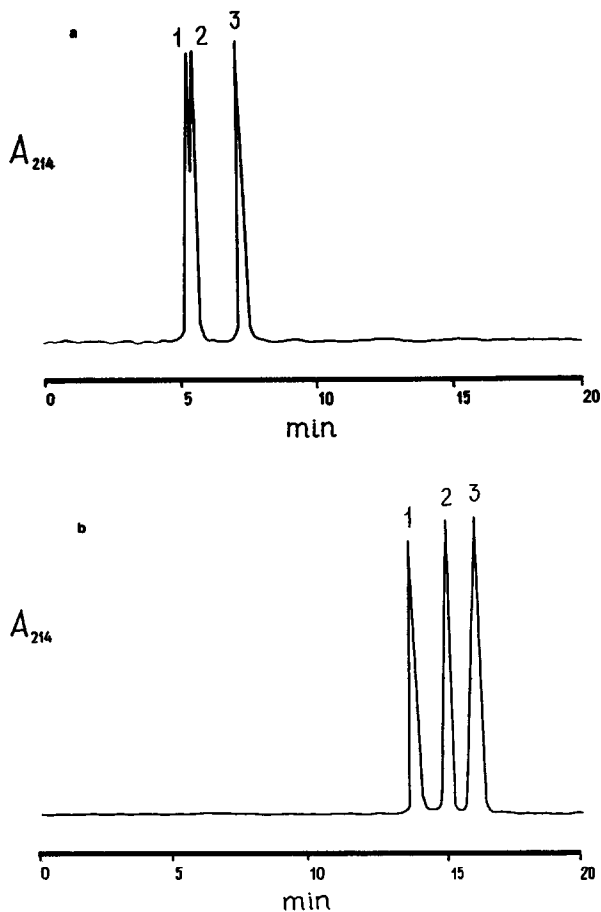
Fig. 6. Separations of renatured recombinant human proinsulin fusion protein (rPFP-r), recombinant human proinsulin (rHP) and recombinant human insulin (rHI) by (a) CZE and (b) MECC. Peaks were identified by migration times of standards: 1 = rPFP-r; 2 = rHP; 3 = rHI. Analysis was performed using the Applied Biosystems 270A CE system. Conditions: fused-silica capillary supplied by Beckman, I.D. 100 μm, length 52 cm, effective length 40 cm; voltage, 18 kV; temperature, 28°C; buffer, (a) 0.1 M borate (pH 9.3) and (b) 0.1 M borate–0.1 M SDS (pH 9.3); concentration of protein in sample, 1 mg/ml; electrophoretic injection, 5 s/+5 kV; detection, UV absorbance at 214 nm.
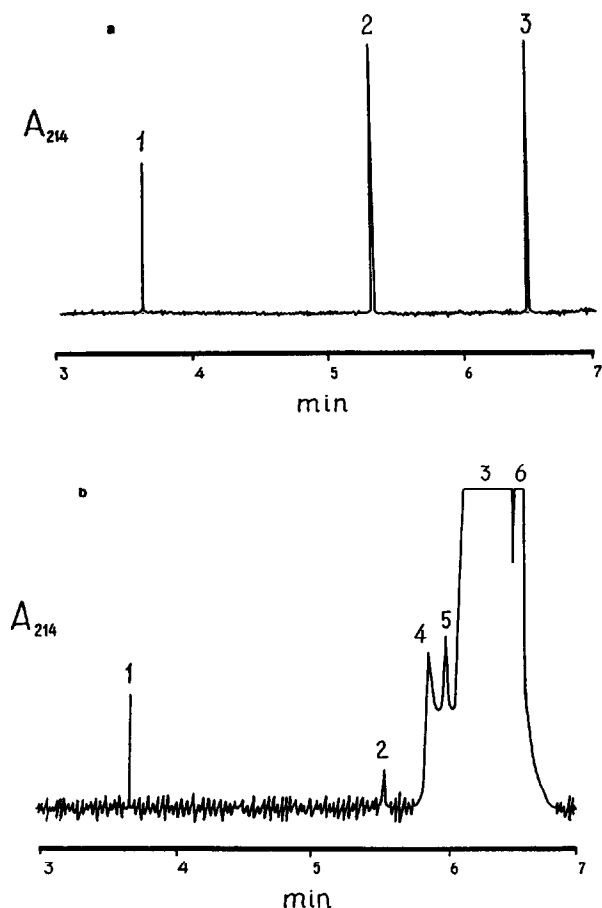
Fig. 7. Separation of recombinant human proinsulin (rHP) and recombinant human insulin (rHI) by CZE under optimum conditions. (a) Model separation. Peaks were identified by migration times of standards: 1 = marker of electroosmotic flow; 2 = rHP; 3 = rHI. (b) Determination of minor impurity of rHP in rHI. Peaks: 1–3 as in (a); 4 = diarginine-(B31–B32)-insulin; 5 = arginil-(AO)-insulin; 6 = desamido-(A21)-insulin (peaks 5 and 6 were identified according to ref. 23). Analysis was performed using the Applied Biosystems 270A CE system. Conditions: fused-silica capillary supplied by Beckman, I.D. 100 μm, length 52 cm, effective length 40 cm; voltage, 20 kV; temperature, 28°C; buffer, 0.03 M Na$_2$HPO$_4$ (pH 11.2); (a) concentration of protein 0.3 mg/ml, electrophoretic injection 0.1 s/+5 kV; (b) concentration of rHP 0.02 mg/ml, electrophoretic injection 8 s/+5 kV; detection, UV absorbance at 214 nm.

paper, described the separation of rHI and the main products of its acid degradation, satisfactorily efficiency and selectivity being achieved.

Proinsulin is also included in the list of pharmacopeial impurities and its concentration is limited more stricly to 0.1%. The efficiencies of seveal thousand TP demonstrated by HPLC in the analysis of proteins lead to the impossibility

of determining this minor impurity owing to the low statistical authenticity of the results. Today slow and expensive immunoassays and PAGE with overloaded track are used for the determination rHP in rHI [4]. This separation by HPCE

has not yet been discussed in the literature. CZE separation under optimized conditions allowed us to determine less than 0.1% of rPH in rPI (Fig. 7b).

In conclusion we want to discuss briefly the potential of CZE. The various advantages of HPCE have often been discussed in the literature, and the combination of practicalness, economy and high resolving power would without doubt ensure a leading role for this method in separation science, if it were not for one great disadvantage, i.e., HPCE is applicable only in the analytical mode. all reports of micropreparative HPCE separations are connected with complicated equipment and a narrow range of application. This is the reason why we cannot today consider HPCE to be a really powerful and independent method. However, in combination with other methods, HPCE can show superior results [22].

The combination of HPCE and HPLC is very easy, because the methdos are similar both in theory and in practice.

The application of different methods of SE-, IE- and RP-HPCE and HPCE (CZE and MECC) for the analysis of insulin, insulin-related and non-insulin-related substances have been studied in this work. A combined system of HPLC and HPCE for the step-by-step control of recombinant human insulin production technology has been suggested. The advantages and shortcomings of these methods have been discussed. Chromatographic columns with commercial and specially developed domestic supports for SE-, RP- and IE-HPLC have been used and their effective application for the analysis of products and semiproducts at every stage of the technology concerned has been demonstrated. The combination of optimized methods of SE-, RP-, and IE-HPLC and CZE and MECC can form the basis of a production control system.

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