determining the amino acid sequence of peptides containing a ΔTrp residue. The most important advantage of this method of analyzing the ΔTrp peptides in comparison with chemical methods of analysis is the absence of the side reaction characteristic for ΔTrp, the rapidity and ease of obtaining the results, and the possibility of working with micro amounts of difficulty accessible peptides.

SUMMARY

It has been established that the mass spectra of N-Bz, and N-Ac derivatives of peptides containing α,β-didehydrotryptophan residues have characteristic peaks of ions with m/e 130, 156 (157), and 183; these permit the identification in peptides of the α,β-didehydrotryptophan residue, which is unstable on hydrolysis. In the course of the fragmentation of dehydropeptides by the amino acid route, the formation of the intense peak of the ion of an azlactone of aN-acyl-α,β-didehydrotryptophan is characteristic. The mass-spectrometric method can be used for determining the amino acid sequences of peptides containing α,β-didehydrotryptophan residues.

LITERATURE CITED

1. A. B. Silaev, G. S. Katrukha, Zh. P. Trifonova, and I. G. Sinyavskaya, Antibiotiki, No. 1, 13 (1968).
2. J. S. Sheenan, D. Mania, S. Nakamura et al., J. Am. Chem. Soc., 90, 462 (1968).
3. H. A. Whaley, E. L. Patterson, M. Daun et al., Antimicrob. Agents Chemother., 587 (1966).
4. R. Cardillo, C. Fuganti, G. Gatti et al., Tetrahedron Lett., 3163 (1974).
5. R. Cardillo, C. Fuganti, D. Chiringhelli et al., J. Chem. Soc. Chem. Commun., 778 (1975).
6. G. S. Katrukha, S. N. Maevskaya and A. B. Silaev, Bioorg. Khim., 3, 422 (1977).
7. Zh. P. Trifonova, G. S. Katrukha, and A. B. Silaev, Khim. Prir. Soedin., 790 (1972).
8. M. K. Bakhra, G. S. Katrukha, and A. B. Silaev, Khim. Prir. Soedin., 280 (1971).
9. M. K. Bakhra, The Development of Methods for Synthesizing Peptides Containing Dehdyrotryptophan Residues and their Properties, Author's Abstract of Candidate's Dissertation, Moscow (1973).
10. J. van Heijenoort, E. Bricas, B. Das, et al., Tetrahedron, 23, 3403 (1967).
11. B. Das and É. Lederer, in: New Methods of Analyzing Amino Acids, Peptides, and Proteins, [Russian translation], Moscow (1974), p. 189.

SEPHAROSE 4B—DNP-HEXAMETHYLENEDIAMINE AS A SORBENT FOR THE CHROMATOGRAPHY OF CARBOXYLIC PROTEINASES

V. P. Borovikova, N. N. Tarasova,
G. N. Lavrenova, and V. M. Stepanov

UDC 577.1.156

We have previously reported the production of the sorbent Sepharose 4B—N-(2,4-dinitrophenyl)-hexamethylenediamine and its use for chromatography of porcine pepsin and pepsinogen and of the carboxylic proteinase of *Aspergillus awamori* (aspergillopepsin A) at pH 5.6 [1]. However, as has been shown for sorbents with peptide ligands, under these conditions a considerable contribution to sorption is made by ionic interactions [2]. At pH 5.6, pepsin and proteinases related to it are charged negatively. The addition of amines to Sepharose activated with cyanogen bromide leads to the formation of isourea derivatives which have pK_a values of about 10 [3] and are charged positively under the conditions of chromatography. This creates the prerequisites for ionic interaction between ligand and enzyme. We set ourselves the task of evaluating the contribution of ionic and hydrophobic forces in the interaction of sorbents containing a DNP group with a number of carboxylic proteinases.

Chromatography of a purified preparation of porcine pepsin with a specific activity of 42-46 activity units/optical unit was carried out on the sorbent Sepharose 4B—DNP-hexamethylenediamine having a ligand concentration of 7.1 μmole/ml. At pH 4.5 and 5.6 in 0.1 M acetate

M. V. Lomonosov Moscow State University. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 191-199, March-April, 1979. Original article submitted November 24, 1978.

buffers no ballast proteins free of enzymatic activity were sorbed (Fig. 1 and Table 1). In both cases, a large part of the native pepsin was desorbed by a concentration gradient of sodium chloride, the activity peak appearing in 0.3 M NaCl at pH 4.5 and in 0.35 M NaCl at pH 5.6. It is obvious that in both cases the role of electrostatic interactions is a large one. An increase in the ionic strength led to the screening of the charges and the protein was desorbed from the column. The protein not desorbed by sodium chloride was eluted with 20% isopropanol in 1 M NaCl. After elution with sodium chloride a larger proportion of the enzymes remained on the column at pH 4.5 that at pH 5.6. It may be assumed that the reason for this is that at pH 4.5 the hydrophobic interaction plays a larger role. It is possible that the conformation of the pepsin molecule under these conditions is such that its hydrophobic zones are more accessible. It is not excluded that this may be due to the presence close to the hydrophobic binding zone of a carboxy group with a pK value between 4.5 and 5.6 the dissociation of which worsens the conditions for the formation of hydrophobic contacts. When sorbents with a higher concentration of ligand (about 11 μmole/ml) were used, an intensification of sorption was observed. The enzyme could not be eluted even with 1 M sodium chloride, and 20-30% isopropanol was necessary for its desorption.

At pH 2.1, the isoelectric point of the pepsin, in 0.05 M KCl—HCl buffer a considerable part of the enzyme — about 32% of the total amount of protein and 25% of the activity — was eluted with the initial buffer and at the same time it was separated from ballast impurities (Fig. 2a and Table 1). A large part of the enzyme was desorbed by a concentration gradient of isopropanol, which corresponds to the assumption of a purely hydrophobic nature of the binding of the pepsin, neutral as a whole at the given pH, by the positively-charged sorbent. The fall in the yield with respect to activity is due to the autolysis of the enzyme which takes place actively at pH 2.1.

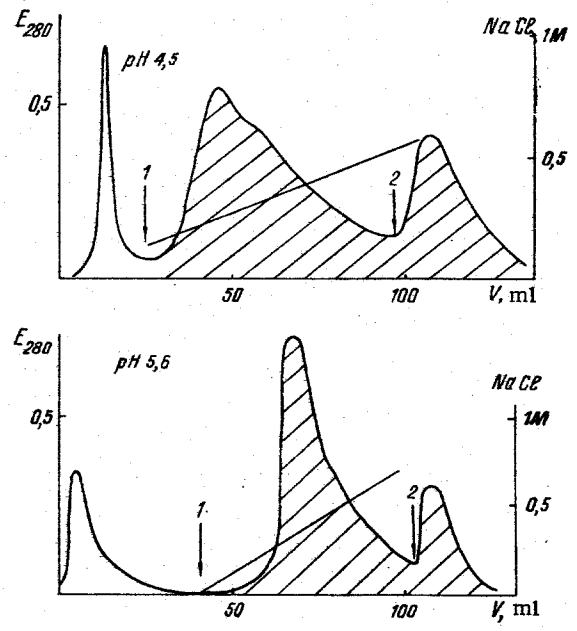

Fig. 1. Chromatography of a purified preparation of porcine pepsin on Sepharose 4B—DNP-hexamethylenediamine (ligand concentration 7.1 mole/ml) at pH 4.5 and 5.6. Pepsin (20 mg in 2 ml of 0.1 M acetate buffer, pH 4.5 or 5.6) was deposited on 2 ml of sorbent. The arrows show the beginning of elution: 1) with a concentration gradient of NaCl (0-1 M); 2) with 20% isopropanol in 1 M NaCl. Here and below, the peaks containing the active enzyme are hatched.

TABLE 1. Chromatography of Porcine Pepsin on the Sorbent 4B—DNP-Hexamethylenediamine

Conditions of chromatography	Not sorbed	Elution with		
		initial buffer	NaCl (0–1M)	isopropanol (0-30%)
0.1 M Acetate buffer, pH 5.6	0/10(0)	0/0(0)	80/62(56)	2/3(9)
0.1 M Acetate buffer, pH 4.5	0/9(0)	0/0(0)	66/45(56)	20/34(24)
0.05 M KCl—HCl buffer, pH 2.1	0/16(0)	25/32(52)	0/0(0)	41/54(30)
0.05 M glycine—HCl buffer, pH 2.1	0/15(0)	52/30(80)	0/0(0)	31/31(42)

Note. Amount of ligand 7.1 μmole/ml; volume of sorbent 2 ml: 20 mg of protein deposited. The numerator gives the activity yield (%) and the denominator the amount of protein in the fraction (% on the total amount of protein deposited on the column), while the specific activity (activity units/optical unit) is given in parentheses.

At the same pH, but in 0.05 M glycine—HCl buffer the quantitative results were different. The pepsin was again separated from the ballast and was eluted when the column was washed with the initial buffer (Fig. 2b and Table 1). However, the proportion of the enzyme eluted by the initial buffer was twice as great as in the preceding experiment which shows a weaker interaction of the pepsin with the sorbent. The difference between sorption in the KCl-containing buffer and in the glycine-containing buffer is apparently the result of competing binding of the pepsin with the glycine present in the buffer.

The presence of a salt (NaCl) at pH 2.1 not only does not cause the desorption of the protein but actually strengthens its binding. Thus, in the presence of 1 M NaCl in the glycine-containing buffer and in the KCl—HCl buffer pepsin was strongly sorbed by the column (Fig. 2c and d). Elution was achieved with isopropanol (≈20%). An intensificaiton of hydrophobic interactions with an increase in ionic strength has been described in the literature (see, e.g., [4]) and is explained by changes in the structure of the water which favor hydrophobic binding.

Chromatography in 0.05 M glycine—HCl buffer, pH 2.1 was used to purify a commercial preparation of porcine pepsin. The ballast proteins not sorbed by the column amounted to 46% of the total weight of the deposited protein. The pepsin peak eluted by the initial buffer contained 30% of the total protein and 71% of the activity. Its specific activity had risen to 58 activity units/optical unit. With 15% isopropanol in 0.05 M glycine—HCl buffer, pH 2.1, 14% of the total protein and 28% of the activity was desorbed; the specific activity of this fraction was 48 activity units/optical unit. Thus, 2.3-fold purification of the preparation was achieved. Chromatography on Sepharose 4B—DNP-hexamethylenediamine in glycine buffer at pH 2.1 can be recommended as an effective method of purifying porcine pepsin.

As the matrix, in place of Sepharose it is possible to use the more stable Ultrogel [5]. Chromatography of purified and commercial preparations of porcine pepsin on the sorbent Ultrogel—DNP-hexamethylenediamine at pH 5.6 gave results similar to those described above.

To elucidate the role of the carbohydrate chain of the ligand, we obtained Sepharose 4B—DNP-ethylenediamine with ligand concentrations of 5.4 and 3.2 μmole/ml and performed the chromatography of a purified preparation of porcine pepsin. At pH 2.1 the pepsin was not retained by the column and issued together with the ballast impurities (Table 2). At pH 4.5, the protein was sorbed on the column. In a sodium chloride gradient the bulk of the protein

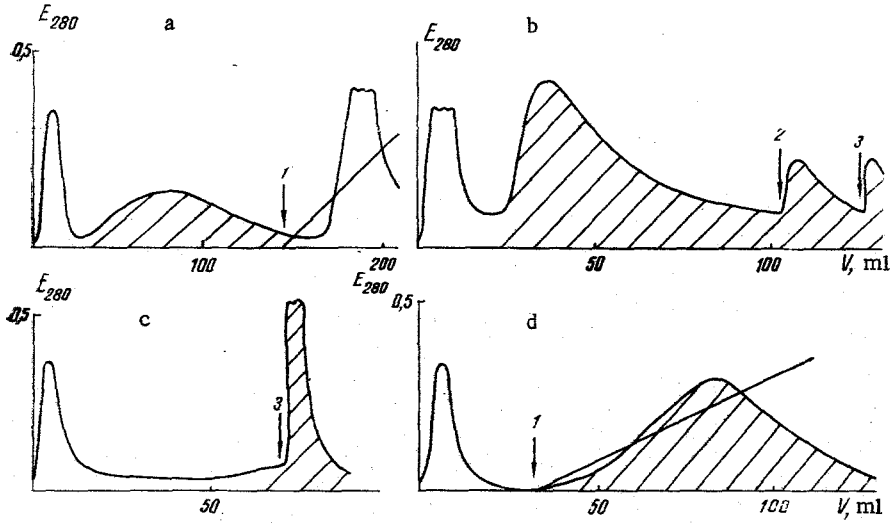

Fig. 2. Chromatography of a purified preparation of porcine pepsin on Sepharose 4B—DNP-hexamethylenediamine (containing 7.1 mole of ligand per ml) at pH 2.1 in 0.05 M KCl—HCl buffer (a), in 0.05 M glycine—HCl buffer (b), in 0.05 M KCl—HCl buffer in the presence of 1 M NaCl (c), and in 0.05 M glycine—HCl buffer in the presence of 1 M NaCl (d). 20 mg of protein in 12 ml of buffer was deposited on 2 ml of sorbent. The arrows show the beginning of elution: 1) with a concentration gradient of isopropanol (0-40%); 2) with 10% isopropanol; 3) with 20% isopropanol.

TABLE 2. Chromatography of Porcine Pepsin on the Sorbent Sepharose 4B—DNP-Ethylenediamine*

Conditions of chromatography	Not sorbed	Elution with	
		NaCl (0–1 M)	25% isopropanol
Concentration of ligand 5.4 μmole/ml (volume of sorbent 3 ml; 20 mg of protein with a specific activity of 22-28 activity units/optical unit was deposited)			
0.05 M KCl—HCl buffer, pH 2.1	85/75 (24)	—	—
0.1 M acetate buffer, pH 4.5	0/7 (0)	92/80 (33)	8/12 (20)
0.1 M acetate buffer, pH 5.6	0/9 (0)	76/80 (27)	8/11 (13)
Ligand concentration 3.2 μmole/ml (volume of sorbent 2 ml; 20 mg of protein with a specific activity of 42-46 activity units/optical unit was deposited)			
0.05 M KCl—HCl buffer, pH 2.1	83/94 (40)	—	—
1 M KCl in 0.05 M KCl—HCl buffer, pH 2.1	85/97 (37)	—	—
0.1 M acetate buffer, pH 4.5	0/10 (0); 17/21 (40)†	79/54 (64)	2/9,5 (42)
0.1 M acetate buffer, pH 5.6	52/50 (47)	41/39 (48)	0/5 (0)

*See Note to Table 1.
†Eluted by the initial buffer before the addition of NaCl.

was eluted at a solvent concentration of 0.35 M, the specific activity in the peak amounting to 33 activity units/optical unit. When 25% isopropanol was used, 12% of the total protein, with a specific activity of 20 activity units/optical unit, was eluted. At pH 5.6 in 0.1 M acetate buffer the interaction of the pepsin with the sorbent was weakened, and almost the whole of the protein was desorbed in a sodium chloride gradient at a salt concentration of 0.25 M. A decrease in the amount of ligand in the sorbent to 3.2 μmole/ml led to a further weakening of the bond of the enzyme with the sorbent.

A comparison of the results of chromatography on this sorbent with the results obtained on Sepharose 4B—DNP-hexamethylenediamine shows that a shortening of the carbohydrate chain of the ligand by four methylene units substantially weakens the interaction of pepsin with the sorbent. The reason for this may be both a decrease in the hydrophobicity of the ligand and a worsening of the access of the center of binding of the DNP group in the enzyme to the ligand through the shortening of the molecular insertion.

Like pepsin, the acid proteinase of the fungus *Aspergillus awamori* (aspergillopepsin A) belongs to the class of carboxylic proteinases [6]. This enzyme has an isoelectric point of 3.99 and is analogous to pepsin in structure and functional properties. In order to study the influence of ionic and hydrophobic interactions on the chromatography of aspergillopepsin A a purified preparation of the enzyme with a specific activity of 25 activity units/optical unit was chromatographed on Sepharose 4B—DNP-hexamethylenediamine with a ligand concentration of 7.1. μmole/ml. At pH 2.1 in 0.05 M glycine—HCl buffer, the enzyme issued from the column completely on deposition. This is explained by the fact that under these conditions both the protein and the sorbent ($pK_a \sim 10$) [3] are positively charged. At pH 4.0 in 0.1 M acetate buffer the proteinase was sorbed completely on the column and elution was achieved with a concentration gradient of sodium chloride at a concentration of the salt of 0.13 M. Consequently, the presence of the salt worsens the binding of the enzyme with the sorbent at the isoelectric point and does not improve it as was the case with porcine pepsin at its isoelectric point of pH 2.1. The specific activity of the purified enzyme amounted to 30 activity units/optical unit. At pH 5.6 the enzyme was again sorbed completely on the column and was eluted completely in a concentration gradient of sodium chloride at a salt concentration of 0.2 M. The degree of purification was the same at pH 4.0. The chromatography of a preparation with a specific activity of 2.5 activity units/optical unit in a concentration gradient of sodium chloride permitted a fourfold purification of the enzyme.

Thus, aspergillopepsin A possesses a distinct affinity for a 2,4-dinitrophenyl sorbent, but this is less pronounced than that of porcine pepsin. An increase in ionic strength reduces the binding of the enzyme with the sorbent at all pH values. Chromatography on this sorbent can be used for purifying aspergillopepsin A.

A 2,4-dinitrophenyl sorbent has been used previously in our laboratory to isolate a mixture of bovine pepsin and chymosin from an industrial preparation of rennet [7]. The separation of these two enzymes with similar properties was achieved by chromatography on Sepharose 4B—ribonuclease and Sepharose 4B—(ε-aminocaproyl-D-phenylalanine methyl ester) [7]. We have attempted to use the sorbent Sepharose 4B—DNP-hexamethylenediamine, which is more accessible preparatively, for separating these enzymes. The results of the separation of these enzymes obtained in biospecific chromatography on Sepharose 4B—(ε-aminocaproyl-D-phenylalanine methyl ester) at pH 5.6 of an industrial preparation of rennet are shown in Table 3. The two enzymes possess milk-clotting activities that are considerable and close to one another, but pepsin hydrolyzes hemoglobin at pH $\sim$ 2 far more effectively then chymosin. Consequently, the efficiency of the separation of these enzymes was determined in relation to milk-clotting and proteolytic activity (relative activity).

At pH 4.5, the isoelectric point of chymosin, both pepsin, which bears a negative charge under these conditions, and chymosin, the total charge of which is equal to zero, are sorbed on the column and are eluted without separation by a concentration gradient of sodium chloride at a salt concentration of 0.75 M and, partially, by 20% isopropanol in 1 M NaCl. At pH 3.0 (10^{-3} M HCl) the positively charged chymosin is repelled by the matrix, which has a charge of the same sign, while nothing interferes with the strong sorption of pepsin. In this way the separation of the proteins is achieved, but the chymosin issues from the column together with a ballast material and its purification requires rechromatography. This can be avoided if the deposition of the mixture of enzymes is carried out at pH 5.6. Under these conditions, both enzymes are sorbed, and the inactive ballast impurities issue from the column. Elution of the chymosin is achieved with 10^{-3} M HCl, pH 3.0. The ballast proteins are desorbed with 1 M sodium chloride, and the pepsin with 20% isopropanol in 1 M NaCl.

TABLE 3. Chromatography of a Mixture of Bovine Pepsin and Chymosin on Sepharose 4B–DNP-Hexamethylenediamine

pH	Not sorbed	Elution with		
		10^{-3}M HCl, pH 3.0	NaCl (0–1M)	20% isopropanol
3.0	$\frac{43}{51}$(2740)	–	$\frac{0}{14}$(0)	$\frac{32}{40}$(300)
4.5	$\frac{0}{14}$(0)	–	$\frac{43}{46}$(540)	$\frac{31}{24}$(590)
5.6	$\frac{0}{10}$(0)	$\frac{32}{34}$(3420)	$\frac{0}{12}$(0)	$\frac{66}{56}$(480)

Note. Concentration of ligand 7.1 μmole/ml. The numerator gives the yield with respect to milk-clotting activity (%) and the denominator the amount of protein in the peak (% of the total amount of protein deposited on the column), while the relative activity is given in parentheses.

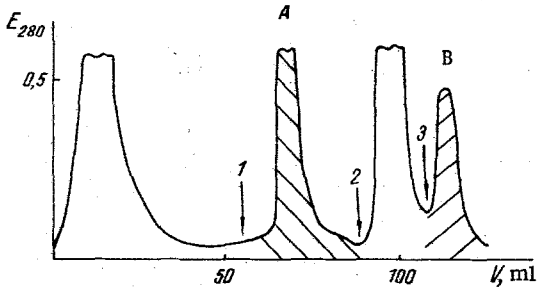

Fig. 3. Chromatography of an industrial preparation of rennet on Sepharose 4B–DNP-hexamethylenediamine (concentration of ligand 7.1 mole/ml). 0.8 g of the preparation in 12 ml of 0.1 M acetate buffer (pH 5.6) was deposited on 2 ml of sorbent. The arrows show the beginning of elution: 1) with 10^{-3} M HCl, pH 3.0; 2) with 1 M NaCl; 3) with 20% isopropanol in 1 M NaCl; A) chymosin; B) pepsin.

A similar experiment was performed with an industrial preparation of rennet (Fig. 3). The initial buffer, with pH 5.6, eluted inactive impurities amounting to 60% of the total amount of protein deposited on the column. The chymosin fraction contained 5% of the total protein, 60% of the milk-clotting activity, and 100% of the hemoglobin-hydrolyzing activity, the relative activity being 5130. The ballast proteins, amounting to 24% of the total protein, were eluted with 1 M NaCl. The peak of the pepsin issuing when the column was washed with 20% isopropanol in 1 M NaCl contained 4% of the total proteins, 40% of the milk-clotting activity, and 67% of the hemoglobin activity, the relative activity being 560. Chromatography led to a 20-fold purification of the chymosin and a 60-fold purification of the bovine pepsin. Thus, bovine pepsin and chymosin, like porcine pepsin and asperigillopepsin A, possess affinity for the DNP sorbent. The chromatography of a commercial preparation of rennet on Sepharose 4B–DNP-hexamethylenediamine performed in the manner described above can serve as an effective method of purifying both enzymes and it simultaneously permits their separation. The separa-

tion of pepsin and chymosin illustrates the possibility of the use of ionic interactions for increasing the efficiency of the process.

EXPERIMENTAL

Enzymes. The porcine pepsin was a commercial preparation obtained from the Moscow meat combine with a specific activity of 25 activity units/optical unit, and we also used an enzyme purified on DEAE-cellulose [8] with a specific activity of 42-46 activity units/optical unit; samples of aspergillopepsin A with a specific activity of 25 and 2.5 activity units/optical unit [6] were used; the rennet had a specific activity with respect to milk clotting of 820-1060 activity units/optical unit and with respect to the cleavage of hemoglobin of 0.2-0.5 activity units/optical unit: we also used a mixture of bovine pepsin and chymosin isolated by chromatographing rennet on Sepharose 4B—(ε-aminocaproyl-D-phenylalanine methyl ester) [7].

Determination of the Activities of the Enzymes. Proteolytic activity was determined from the cleavage of hemoglobin [9]. As the unit of activity we took that amount of enzyme which, under the standard conditions of the determination, cleaved such an amount of hemoglobin that the optical density of a hydrolyzate at 280 nm after precipitation with trichloroacetic acid was 1.

Milk-clotting activity was determined by Alekseenko's method [10] and was calculated by means of the formula

$$\text{Activity (units per ml)} = 2000/t,$$

where t is the clotting time in sec.

Sorbents. Sepharose was activated with cyanogen bromide by the method of Porat et al., [11]. At 5-10°C with stirring, a solution of 0.5 g of cyanogen bromide in 5 ml of water was added to a suspension of 5 g of moist Sepharose 4B (Pharmacia, Sweden) in 7.5 ml of 5 M phosphate buffer, pH 11. The cyanogen bromide was previously dissolved in 0.25 ml of acetonitrile, and then the solution was diluted with water to 5 ml. After activation at 5-10°C for 10 min, the activated Sepharose was washed at 4°C with distilled water to neutrality and then with 50 ml of 0.1 M $NaHCO_3$ and 50 ml of 50% dimethylformamide in 0.1 M $NaHCO_3$, pH 10. The activated Sepharose was added to 10 ml of a 30 mM solution of the hydrochloride of mono-DNP-hexamethylenediamine [1] in a mixture of dimethylformamide and 0.1 M $NaHCO_3$ (1:1), pH 10, and the mixture was stirred at room temperature for 12 h. The sorbent was washed with 50% aqueous dimethylformamide, 0.001 N HCl, and water, and then with all the solvents that it was proposed to use for chromatography until the wash-waters no longer absorbed at 360 nm.

The following sorbents were obtained similarly: Ultrogel—DNP-hexamethylenediamine (LKB Ultrogel AcA 34 was used) and Sepharose 4B—DNP-ethylenediamine. The amounts of ligand in the sorbents were determined from the absorption at 360 nm of a solution of the sorbent in trifluoroacetic acid [1].

Chromatography on the Sorbent Sepharose 4B—DNP-hexamethylenediamine Containing 7.1 μmole of Ligand per ml. A solution of the enzyme in a buffer with the appropriate pH value was deposited on 2 ml of sorbent. The column was washed with the initial buffer until the eluate no longer absorbed at 280 nm. The enzyme was desorbed successively with a concentration gradient of sodium chloride (0-1 M) and with 15-25% isopropanol in 1 M NaCl in the initial buffer. Fractions with a volume of 3 ml were taken, and in each the UV absorption at 280 nm was measured. The proteolytic activities of the fractions that contained protein were determined.

On the column were deposited: 20 mg of porcine pepsin with a specific activity of 42-46 activity units/optical unit in 12 ml of buffer solution, pH 2.1, or in 2 ml of buffer solution, pH 4.5 or 5.6; 40 mg of a commercial preparation of porcine pepsin with a specific activity of 25 activity units/optical unit in 12 ml of 0.05 M glycine—HCl buffer, pH 2.1; 10 mg of aspergillopepsin A with specific activity of 25 activity units/optical unit in 1 ml of buffer (deposited on 0.7 ml of sorbent); 200 mg of aspergillopepsin A with a specific activity of 2.5 activity units/optical unit in 1.5 ml of 0.1 M acetate buffer, pH 4.0; 10 mg of a mixture of bovine pepsin and chymosin with a specific milk-clotting activity of 9500-11,000 activity units/optical unit and hemoglobin-hydrolyzing activity of 16-18 activity units/optical unit and a relative activity of 580-610 [7] in 10 ml of 0.1 M acetate buffer, pH 5.6 pH values of 4.5 and 3.0 being established by the addition of HCl; and 0.8 g of an industrial preparation of rennet with a specific milk-clotting activity of 920 activity units/optical unit and

a hemoglobin activity of 1.0 activity unit/optical unit in 12 ml of 0.1 M acetate buffer, pH 5.6. Chromatography on the Sepharose 4B—DNP-ethylenediamine sorbent was carried out under the same conditions.

SUMMARY

1. The chromatography of the carboxylic proteinases porcine pepsin, aspergillopepsin A, and chymosin on the hydrophobic sorbent Sepharose 4B—DNP-hexamethylenediamine has been studied. It has been shown that the nature of the binding of the proteinases with the sorbent depends on the pH.

2. A shortening of the length of the carbohydrate chain of the ligand by four methylene units substantially weakens the interaction of pepsin with the sorbent.

3. With chymotrypsin and pepsin as examples, the possibility has been shown of using ionic effects for separating these enzymes.

LITERATURE CITED

1. V. M. Stepanov, G. I. Lavrenova, V. P. Borovikova, and C. N. Balandina, J. Chromatogr., 104, 373 (1975).
2. M. M. Chernaya, K. Adli, G. I. Lavrenova, and V. M. Stepanov, Biokhimiya, 41, 732 (1976).
3. R. Jost, T. Miron, and M. Wilchek, Biochem. Biophys. Acta, 362, 75 (1974).
4. G. I. Boellgast and W. H. Fishman, Biochem. J., 141, 103 (1974).
5. G. S. Doley, M. J. Harvey, and P. D. G. Dean, FEBS Lett., 65, 87 (1976).
6. G. G. Kovaleva, M. P. Yusunova, E. N. Lysogorskaya, G. N. Balandian, and V. M. Stapanov, Biokhimiya, 42, 534 (1977).
7. V. M. Stepanov, G. I. Levrenova, K. Adli, M. V. Gonchar, G. N. Balandina, M. M. Slavinskaya, and A. Ya. Strongin, Biokhimiya, 41, 294 (1976).
8. V. M. Stepanov and T. I. Greil', Biokhimiya, 28, 540 (1963).
9. M. L. Anson, J. Gen. Physiol., 22, 79 (1938).
10. L. P. Alekseenko, in: Modern Methods in Biochemistry, V. N. Orekhovich (ed.), [in Russian], Moscow (1968), p. 120.
11. J. Porat, K. Aspberg, H. Drevin, and R. Axen, J. Chromatogr. 86, 53 (1973).

INVESTIGATION OF THE CIRCULAR DICHROISM SPECTRA OF BROMINE-SUBSTITUTED NUCLEIC ACID FRAGMENTS

I. CIRCULAR DICHROISM SPECTRA OF 8-BROMINE-SUBSTITUTED PURINE NUCLEOTIDES

G. V. Siderov, A. F. Usatyi, and N. F. Masoedov

UDC 547.857:547.65

A number of communications [1-4] have been devoted to the study of the optical activity of natural derivatives of purine and pyrimidine compounds. The information given in them permits an explanation of the features of the electronic structures of the compounds investigated and, furthermore, provides the possibility of quantitatively connecting a change in optical properties with a change in conformational states [5-7]. The introduction of a halogen atom into the heterocyclic base of a purine or a pyrimidine nucleotide changes the energy of the electronic transitions and must also change the conformation of the compound as compared with the initial, unhalogenated, state. This change is connected mainly with the angle of rotation of the heterocyclic base around the glycosidic bond (φ_{CN}). According to Donohue and Trueblood [8], the compound has the anti conformation when $\varphi_{CN} = -30\pm45°$ and the syn conformation when $\varphi_{CN} = 150\pm45°$.

Institute of Molecular Genetics, Academy of Sciences of the USSR, Moscow.

Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 199-204, March-April, 1979. Original article submitted November 30, 1978.

0009-3130/79/1502-0168$07.50