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Note

Use of the rapid octadecasilyl column method for purification of ^{125}I-bovine pancreatic polypeptide compared with conventional methodology

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Many procedures have been described for preparation of radioiodine-labeled bovine pancreatic polypeptide for radioimmunoassay. Following iodination, it is necessary to purify the labeled hormone from unreacted radioiodide and damaged components. A wide variety of purification methods have been employed including gel filtration on Sephadex G-50 (refs. 1 and 2), starch gel or polyacrylamide gel electrophoresis[3] and microfine silica (QUSO)[4]. All of these purification methods are efficient and provide sufficient assay sensitivity, but they are also relatively laborious and time-consuming. The present communication describes the purification of labeled bovine pancreatic polypeptide by adsorption to and elution from octadecasilyl silica cartridges (Sep-Pak C_{18}) and a comparison of this method with the previously employed methods.

EXPERIMENTAL

Materials

Rabbit anti-bovine pancreatic polypeptide antiserum (615-R110-146-10) and bovine pancreatic polypeptide for labelling and standard were generously provided by Dr. Ronald Chance, Lilly Research Laboratory (Indianapolis, IN, U.S.A.). Sep-Pak C_{18} cartridges were purchased from Waters Assoc. (Milford, MA, U.S.A.), microfine silica (QUSO 32) from Philadelphia Quartz (Philadelphia, PA, U.S.A.) and all other chemicals from Fisher Scientific (NJ, U.S.A.).

Methods

Bovine pancreatic polypeptide was dissolved in water (pH 2.3) and stored at −20°C until labelling by the method of Hunter and Greenwood[5]. A volume of 5 μl of ^{125}I-labeled sodium iodide (100 mCi/ml) was added to 20 μl phosphate buffer (pH 7.5), followed by the rapid sequential addition of 3 μl bovine pancreatic polypeptide (0.5 mg/ml), 10 μl of a chloramine-T solution (containing 5.4 mg/ml phosphate buffer), 20 μl of a sodium metabisulfite solution (4.8 mg/ml phosphate buffer) and 20 μl bromphenol blue-stained human plasma.

Iodination tube contents were mixed by gently bubbling air through pipettes

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after each addition. The labeled bovine pancreatic polypeptide was then diluted with 100 μl of phosphate buffer, divided into four portions, and immediately purified by one of the following methods.

Microfine silica (QUSO) method. 40 μl of the iodination mixture was added to 1 ml plasma containing 5 mg microfine silica granules (QUSO) and centrifuged at 2000 rpm for 15 min. The supernatant was discarded before the silica pellet was washed with 3 ml distilled water three times. The adsorbed hormone was eluted by vortexing with 0.5 ml of 0.18 *M* HCl in 75% ethanol and 1.5 ml distilled water before centrifugation. The supernatant containing the purified labeled hormone was decanted.

Starch gel method. Starch gel was prepared by the method of Smithies[6]. Starch gel electrophoresis using a boric acid buffer at pH 8.0 was run at 150 V for 18 h. The ^{125}I-bovine pancreatic polypeptide migrates less anodally than albumin. After completion of electrophoresis, gel fractions corresponding to zones of radioactive hormone as determined by autoradiography were excised and frozen at −10°C for 3 h. After thawing, 1 ml of veronal buffer (0.02 *M*) containing 0.25% human serum albumin was added to each gel fraction, and each fraction was then individually squeezed for hormone recovery.

Sep-Pak C_{18} cartridge method. C_{18} cartridges were prepared by the sequential washing with 2.5 ml of 100% methanol and then 10 ml of distilled water. Adsorption to C_{18} cartridges was performed using different concentrations of alcohol (see Table I). 2 ml of 0.01 *M* HCl in 40% ethanol was added to the iodination mixture prior to application to the cartridge. The cartridge was washed with 2 ml of 0.01 *M* HCl in 40% ethanol followed by 10 ml distilled water. Labeled bovine pancreatic polypeptide was eluted from the C_{18} cartridge with 2 ml of 0.01 *M* HCl in 50% ethanol in four 0.5-ml fractions.

Sequential microfine silica and Sep-Pak C_{18} cartridge method. Iodination mixture was sequentially purified on microfine silica initially and then repurified by the C_{18} cartridge method, using both methods as previously described.

Sephadex G-50 column method. The labeled bovine pancreatic polypeptide was purified according to methods previously described by Chance *et al.*[1] using Sephadex G-50 fine packed in a 50 × 0.4 cm I.D. column. The purified label was obtained from the main peak.

TABLE I

ADSORPTION AND ELUTION PATTERN OF ^{125}I-BOVINE PANCREATIC POLYPEPTIDE IN VARYING PERCENTAGES OF ETHANOL

The acid concentration (0.01 *M* HCl) remained constant.

Ethanol (0.01 M HCl) (%)	*Adsorption (%)*	*Elution (%)*
10	97	3
20	96	4
30	95	4
40	93	20
50	61	80
60	17	80
70	14	80

Radioimmunoassay. Radioimmunoassay was performed using Antiserum No. 615-R110-146-10 at a final dilution of $1:6 \cdot 10^6$. The assay incubation time was six days with a three-day delayed addition of the labeled tracer to the incubation mixture. The standard diluent for the assay was 0.02 *M* veronal buffer (pH 8.6) with 2.5 mg human serum albumin per ml. Separation of bound from free ^{125}I-bovine pancreatic polypeptide was effected by addition of 0.1 ml pooled blood bank plasma and then 0.2 ml of 100 mg charcoal per ml 0.02 *M* veronal buffer to the incubation mixture. Blood samples were collected fasting and at 120 min after a protein test meal. Blood for hormone determination was drawn into heparinized tubes, and plasma was separated and stored at −20°C until studied.

RESULTS

Following iodination of bovine pancreatic polypeptide the labeled hormone was adsorbed to and eluted from the C_{18} cartridge. Adsorption of purified labeled hormone was greater than 90% from 10 to 40% HCl–ethanol, and elution greater than 80% of the label was accomplished at HCl–ethanol concentrations greater than 50% (Table I). If one applies the iodination mixture at the highest HCl–ethanol concentration compatible with label adsorption (*i.e.*, 40%) and elutes with the lowest HCl–ethanol concentration consistent with good label recovery (*i.e.*, 50%), then one may optimize label recovery while undesirable products of iodination will either have passed through the cartridge before or remain adsorbed to the cartridge after elution of the labeled peptide. The specific activity of the various bovine pancreatic polypeptide tracers was comparable as judged by a similar displacement in the bound/free (B/F) ratio by equal amounts of radioactivity in the presence of constant antibody concentrations. After three separate iodinations, all five purification methods (starch gel electrophoresis, microfine silica, C_{18}, sequential microfine silica C_{18} and Sephadex G-50) showed parallel standard curves with no significant differences in either sensitivity (as compared by 10% and/or 50% reduction in percent bound) or range (Fig. 1). The minimum detectable concentration (*i.e.*, decrease of 10% in bound/free (B/F) ratio) was 2 pg bovine pancreatic polypeptide per ml and the 50% displacement of the tracer was 10 pg/ml.

To evaluate stability of the different purification methods, we compared the non-specific and antibody specific binding immediately after purification, and then again at three, six and twelve weeks. As judged from two separate iodinations, starch gel electrophoresis, microfine silica, C_{18}, sequential microfine silica/C_{18} and Sephadex G-50 purified tracers were equally stable for six weeks. However, from a single iodination, it would appear that the C_{18} and sequential microfine silica/C_{18} demonstrated greater stability at twelve weeks (Table II). In five healthy subjects fasting and meal stimulated plasma pancreatic polypeptide levels were measured employing tracers purified by the aforementioned methods. Values obtained were the same regardless of the method of label purification.

DISCUSSION

The method described in the present report is based on the adsorption of peptides to hydrophobic octadecasilyl silica and elution with an aqueous solvent

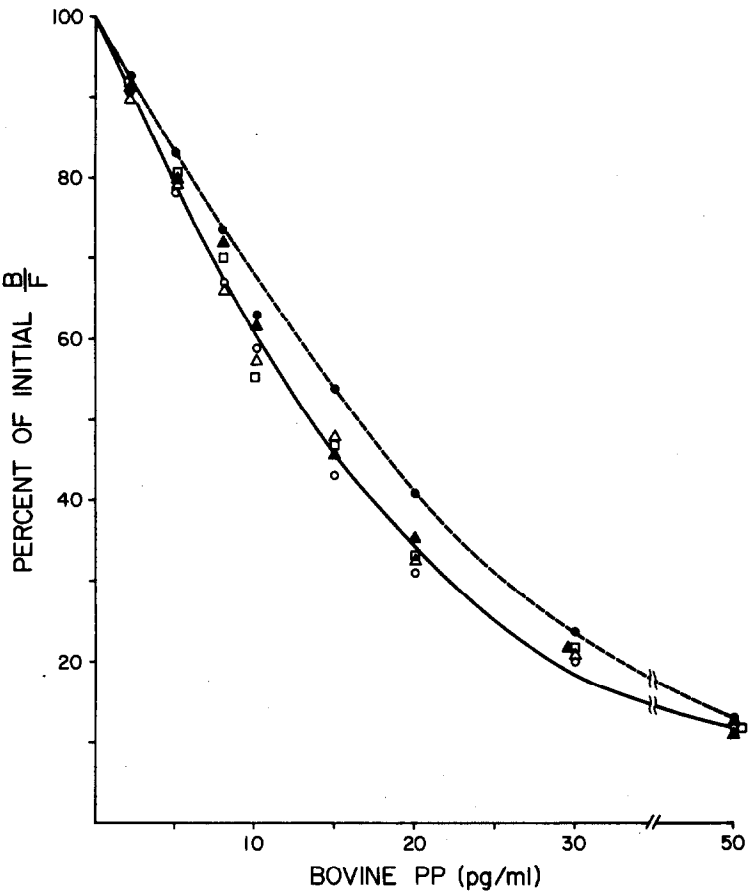

Fig. 1. Comparison of various ^{125}I-bovine pancreatic polypeptide tracers. The curves represent mean values of three assays using labels that were produced from the iodinations in which all labels were compared. All tracers had initial bound/free (B/F) ratios that varied between 0.7 and 1.0. The standard employed was bovine pancreatic polypeptide (bovine PP). ●, Starch gel; ○, C_{18} Sep-Pak; □, QUSO; ▲, QUSO then C_{18} Sep-Pak; △, Sephadex G-50.

mixture containing a relatively hydrophobic component, such as ethanol. The method is similar in principle to silica purification but octadecasilyl silica adsorbs more strongly and extends the adsorption to small peptides[7]. The peptides are quantitatively adsorbed and are capable of being quantitatively eluted in small solvent volumes. The elution position is dependent on the total content of hydrophobic residues and their distribution.

A wide variety of other techniques have been used to separate radioiodinated peptides from unreacted iodide and other impurities. Some well-established methods include Sephadex gel filtration, vertical gel electrophoresis and more recently microfine silica[4]. Chance *et al.*[1] purified the ^{125}I-pancreatic polypeptide by Sephadex G-50 gel filtration or by polyacrylamide gel electrophoresis[3]. Both procedures are relatively laborious and time-consuming. Purification by C_{18} Sep-Pak cartridges takes about 5 min.

TABLE II

MEAN VALUES OBTAINED FROM TWO SEPARATE IODINATIONS FOR BINDING AND NON-SPECIFIC BINDING

	Gel	*Microfine silica*	C_{18}	*Microfine silica* C_{18}	*Sephadex G-50*
Original bound/free %					
3 weeks	78	96	92	94	91
6 weeks	60	87	93	86	72
12 weeks*	50	73	85	82	
Non specific binding %					
1 weeks	7	8	8	8	8
3 weeks	9	13	13	13	13
6 weeks	21	16	16	14	17
12 weeks*	35	22	19	17	

* Data obtained from a single iodination.

The purification method employing C_{18} cartridge yields tracer immuno-reactivity equivalent to that obtained by Sephadex gel filtration, gel electrophoresis or microfine silica. The maximum tracer binding obtained by each of the five methods varies slightly and unpredictably between iodinations but unknown samples assayed against the various standard curves yield values in good agreement.

The technique of purification for ^{125}I-pancreatic polypeptide by C_{18} cartridges is a simple method requiring less than 30 min for the iodination and purification. In addition, repurification of ^{125}I-pancreatic polypeptide can be easily accomplished using C_{18} methodology. Both the C_{18} and sequential microfine silica–C_{18} methods may provide slightly better stability than conventional purification methods. The C_{18} ^{125}I-purification method has proven to be as useful as are the more conventional methods in obtaining a label that can be used in experimental and/or clinical studies of plasma and/or tissue concentrations of pancreatic polypeptide.

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REFERENCES

1 R. E. Chance, N. E. Moon and M. G. Johnson, in B. M. Jaffe and H. R. Behrman (Editors), *Methods of Hormone Radioimmunoassay*, Academic Press, New York, 1979, pp. 658–672.
2 T. W. Schwartz, J. F. Rehfeld, F. Stadil, L. I. Larsson, R. E. Chance and N. Moon, *Lancet*, i (1976) 1102.
3 J. Marco, J. A. Hedo and M. L. Villanueva, *J. Clin. Endocrinol. Metab.*, 46 (1978) 140.
4 M. M. Chen, R. F. Murphy and S. N. Jaffe, *Clin. Chem.*, 7 (1982) 1596.
5 W. M. Hunter and F. C. Greenwood, *Nature (London)*, 194 (1962) 495.
6 O. Smithies, *Adv. Protein Chem.*, 19 (1959) 65.
7 M. P. J. Bennett, A. M. Hudson, L. Kelly, C. McMartin and G. E. Purdon, *Biochem. J.*, 175 (1978) 1139.