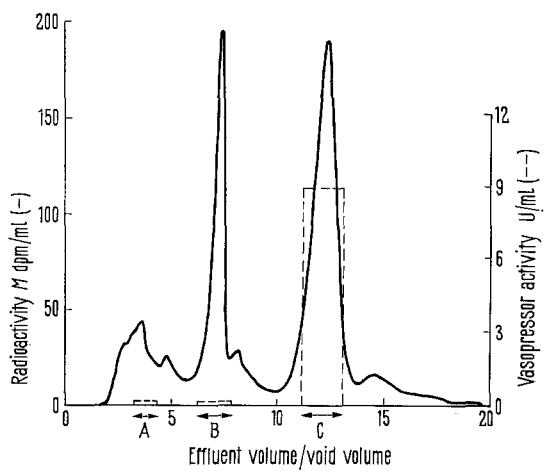
Purification and Stability of Specifically Tritium Labelled Lysine Vasopressin (Tyrosine^2-3-T-lysine^8-vasopressin)

In a previous paper the synthesis of tritium labelled lysine-vasopressin (tyrosine^2-3-T-lysine^8-vasopressin) (T-LVP) with a high specific activity and its purification by gel-filtration (Sephadex G15) was described[1,2]. This hormone has excellent properties for biological studies because a specific labelling limits the number of possible radioactive metabolites in comparison with an unspecific one. Two and a half years later the preparation contained a considerable amount of chromatographically detectable radioactive impurities, originating partly from polymerization, partly probably from radiolysis, while its biological activity had fallen from 57 to 23 IU/ml. The preparation had been stored at 4 °C in a water solution, acidified by acetic acid to pH 4.

The aim of the present work was: (1) to calculate the recovery of radioactivity and to estimate the actual value of the specific labelling in T-LVP after this storage period, (2) to purify the labelled hormone by a more effective method than that described in the paper by SJÖHOLM and CARLSSON[2]. For this purpose we employed CM-Sephadex, which has previously been used for different vasopressin analogues[3,4].

In our procedure we have used a column of 140 cm length, 1.5 cm diameter, filled by 45 g of CM-Sephadex C-25. The total bed volume was 247 ml, the void volume 45 ml. The sample of T-LVP was first evaporated to a small volume in vacuum at 30 °C, then diluted to the original volume and evaporated again. This latter procedure was repeated 3 times to remove tritiated water. The peptide material (in a volume of approx. 5 ml) was then adsorbed on the column and eluted by 0.5 *M* ammonium acetate. This whole procedure was done in a cold room at 4 °C. The fraction volume was 5 ml, flow rate 0.76 ml/min. 200 fractions were collected and the radioactivity was measured in 10 µl of each sample by means of liquid scintillation spectrometry using the dioxane/naphthalene scintillation solution[5]. The elution diagram is demonstrated in the Figure. The first, inhomogenous peak (A) was characterized by a ratio of elution volume to void volume of 3.5, the (B) peak by a ratio of 7.25–7.5, the (C) peak by a ratio of 12.25–13.5. The fractions were pooled (samples A, B, C) according to radioactivity as indicated in the Figure. The pressor activity in samples A, B, and C and in the original sample was assayed on a male rat in urethane anesthesia with a pentolone premedication (Ansolysen®, Pharma Rhodia) and by means of a Statham transducer for blood pressure registration (standard: Vasopressin synth.®, Sandoz). Only fraction C contained vasopressor active material, which amounted to 85% of the pressor activity present in the original sample used for purification. On thin-layer chromatography[6], only one peak of radioactivity was found in this fraction (measured on a window-free chromatogram scanner Nuclear Chicago 4 II Actigraph, model 1032).



Elution diagram from CM-Sephadex C-25 column of tyrosine^2-3-T-lysine^8-vasopressin after $2^1/_2$ years of storage at 4 °C. Conditions see text. Double arrows indicate pooled fractions A, B, C.

The recovery of the radioactivity in the $2^1/_2$ year old preparation of T-LVP is shown in the Table. The specific labelling of T-LVP, calculated from the pressor activity in fraction C and the dpm estimated by means of an internal standard of tritiated toluene was 1.52 ± 0.14 C/m*M*. The specific activity at the time of synthesis was 1.9 C/m*M*[2]. Consequently, the total radioactivity in the active fraction decreased after $2^1/_2$ years of storage to 28%, while the specific radioactivity of T-LVP only fell to 80%. The isotopic exchange reactions, in which T-LVP takes part, seem to be much slower at the concentration used than reactions leading to destruction of vasopressin in other ways[7].

Radioactivity in	Radioactivity in % of (mean or mean ± S.D.) Original sample	Peptide fraction[a]
Water	23.8 ± 3.3	
Fraction A	6.2	7.7
Fraction B	12.4	15.6
Fraction C	27.6 ± 9.7	34.1 ± 8.6

[a] Radioactivity in original sample minus radioactivity in evaporated water.

Zusammenfassung. Tyrosin^2-3-T-Lysin^8-Vasopressin, das vor $2^1/_2$ Jahren synthetisiert worden war, wurde auf CM-Sephadex-C-25-Säulen gereinigt. Die spezifische Radioaktivität des spezifisch markierten Hormons war nur auf 80% gefallen, was einen verhältnismässig kleinen Grad von Isotopaustausch für das Nonapeptid selber anzeigt.

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