Long term stability of lysine vasopressin and of specifically tritiated lysine vasopressin in weakly acidic aqueous solutions[1]

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Summary. Lysine vasopressin tritiated at meta position of tyrosine (1.9 Ci/mmole) decomposes during a long storage period, mainly as a result of radiolysis. The loss of specific activity by isotopic exchange with water is slow. The non-labelled peptide undergoes only very minute decomposition (polymerization). Specific radioactivity declines with a half life of 47.4 months.

In 1966, Sjöholm and Carlsson[2,3] prepared [Tyr(3-^{2}H)^2]-lysine vasopressin (tLVP), a tritiated peptide with the label in a single position in the aromatic ring of tyrosine. The product possessed a specific radioactivity of 1.9 Ci/mmole. It was used, after purification[4], for tissue distribution studies in vivo and in vitro, for binding studies etc. We had the opportunity to follow its decomposition over more than 12 years and to compare this with the long term decomposition of several preparations of nonlabelled lysine vasopressin (LVP). Hoping that such observations may be of interest for those who are dealing with similar radioactively labelled peptides, we would like to describe our results in this short note.

Experimental. LVP and tLVP were stored at 2-4 °C in sterile solution at a concentration of approximately 0.1-0.2 mg of dry peptide substance per ml (initial blood pressure activity was 20-55 IU/ml) in a weakly acidic solution (pH 4.0, acetic acid). For comparison, stability of LVP was also followed at 20 °C. Before measuring the specific radioactivity, a sample of about 10 ml was treated as described earlier (evaporation of tritiated water) and purified on a CM-Sephadex C-50 (or C-25) column[4]. The tLVP-fraction is chromatographically[5] and electrophoretically pure but contains a large amount of ammonium acetate (gradient compound). Desalting was carried out on a Sephadex G 10 (Medium) column, 147 × 14 mm, in 2 M acetic acid which was subsequently removed by repeated evaporation in vacuo at 25 °C. The relative elution volumes for tLVP and salts (measured by conductance) were 1.2 and 1.8, respectively. The recovery of tLVP amounted to 78-91%, depending on the quantity loaded onto the column (higher recovery for small loads). Since the elution volume of tLVP increases with decreasing concentration of acetic acid in the water phase, the separation at lower acid concentrations (0.1 and 1.6 M) was unsatisfactory.

The radioactivity was measured by liquid scintillation spectrometry. The amount of biologically active lysine vasopressin was assayed by a blood pressure bioassay on male rats[4].

Results. Results are summarized in figure 1, a, for tLVP and in figure 1, b, for LVP. The specific radioactivity and the amount of the biologically active substance in tLVP samples, presented as percent of initial values, decrease approximately exponentially with half-lives 47.4 and 29.4 months, respectively. The difference is statistically significant (regression analysis, t-test; $p<0.05$), although the scatter of the former measurement is somewhat higher. Also the ratio of tLVP to total radioactivity expressed as the radioactivity of the tLVP-peak to the loaded radioactivity (sample radioactivity minus radioactivity in evaporate) declines exponentially, with an even shorter half-life than the latter 2 parameters (40.7 months). The radioactive peptide products of tLVP decomposition (loaded radioactivity minus radioactivity in tLVP) display an asymptotic increase (however, almost linear during the first 60 months). After 150 months, 34.2% of total radioactivity was present in the volatile fraction (tritiated water). The half-life of LVP in nonlabelled preparations was 237 months (value obtained by regression analysis of all data in figure 1, b).

Discussion. 4 main processes[6] participate on tLVP decomposition: 1. the selfdecomposition of tritium ('disintegration') which is the primary internal radiation effect, 2. damage caused by primary external and/or secondary radiation effects to the labelled and nonlabelled material present in the solution, 3. chemical effects the origin of which is essentially unconnected with radiation, and 4. isotopic exchange yielding nonlabelled peptide (LVP). The overall decay model can be matched by the scheme in figure 2, where α is the rate constant of isotopic exchange, β and γ are pooled rate constants for tLVP and LVP, respectively, for processes mentioned under 2 and 3 (individual processes could not be differentiated in our experiments), and λ is the known rate constant of tritium disintegration, $\lambda = 0.0047$ month^{-1} (Evans[7]). All rate constants are assumed to be first order. Rough estimates of their values can be based on a simplified mathematical description of this model. The time courses of changes of tLVP (concentration x), LVP (concentration a) and radioactive peptide products (concentration y) are given by the following set of equations

$$x = x_0 e^{-(\alpha+\beta+\lambda)t} \qquad (1)$$

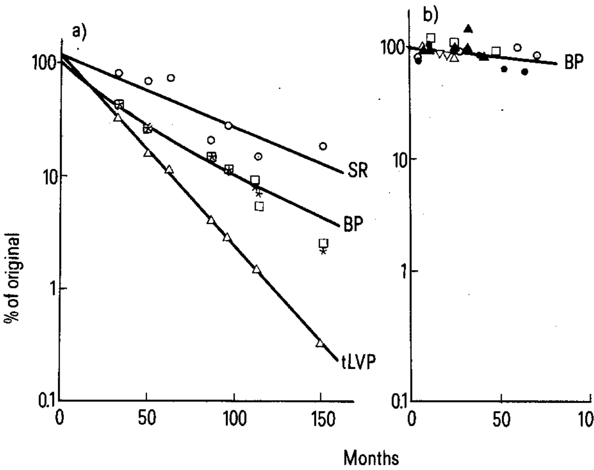

Fig. 1. *a* Time change of specific radioactivity, SR (○), total amount of tLVP (△) and total amount of biologically active substance in blood pressure assay, BP (experimental points □, smoothed data * - see text). Solid lines are single (SR, tLVP) or double exponential fits (BP). *b* Time change of LVP concentration (assayed by rat pressure, BP) in LVP solutions (initial concentration approximately 0.1 nmole/ml) at 4 °C (open symbols) and 20 °C (closed symbols). Various symbols show individual experimental series. Straight line: least square approximation.

$$y = x_0\left(\beta/(\alpha+\beta)\right)\left(e^{-\lambda t} - e^{-(\alpha+\beta+\lambda)t}\right) \tag{2}$$

$$b \equiv a + x = x_0\left(1 - \alpha/(\alpha+\beta+\lambda-\gamma)\right) e^{-(\alpha+\beta+\lambda)t} + \left(a_0 + x_0\alpha/(\alpha+\beta+\lambda-\gamma)\right) e^{-\gamma t} \tag{3}$$

where x_0, a_0 are the corresponding initial concentrations and b stands for the total concentration of biologically active lysine vasopressin (tLVP+LVP). The rate constants α, β can be obtained from the first 2 equations using linear regression applied on a coupled relation

$$y = x_0\left(\beta/(\alpha+\beta)\right) X \tag{4a}$$

where

$$X = x/x_0 - e^{-\lambda t}; \tag{4b}$$

the sum $\alpha+\beta$ was computed from equation (1) using the least square optimalization technique. 2 methods were employed for computation of the constant γ, both applied to b-data: the double exponential fit (eq. 3) according to Foss[8], and direct integration of the rate equation for b. This rate equation is

$$db/dt = (\gamma - \beta - \lambda)\, x - \gamma b \tag{5}$$

and its integrated form is

$$b_t - b_0 = (\gamma - \beta - \lambda)\int_0^t x\, dt - \gamma \int_0^t b\, dt \tag{6}$$

where b_t is the value of b at the time t, and b_0 is its initial value. The values of the integral $\int_0^t x\, dt$ were computed from the exponential approximation (eq. 1; cf. figure 1, a). For the computation of $\int_0^t b\, dt$ a polynomial approximation (4th order) of the time series in b was employed (cf. figure 1, a). The multiple regression analysis applied to equation (6) indicated, as expected, a very significant correlation between b_t and the 2 integrals and allowed an estimate of the rate constants β and γ. The individual rate constants obtained by the procedures described here are as follows: $\alpha = 0.0071$, $\beta = 0.027 \pm 0.004$ (4), $\gamma = 0.018 \pm 0.001$ (3), all in month^{-1}. The constants β and γ are arithmetic means ± SD of data obtained by various computational procedures mentioned above (number of available values in parentheses).

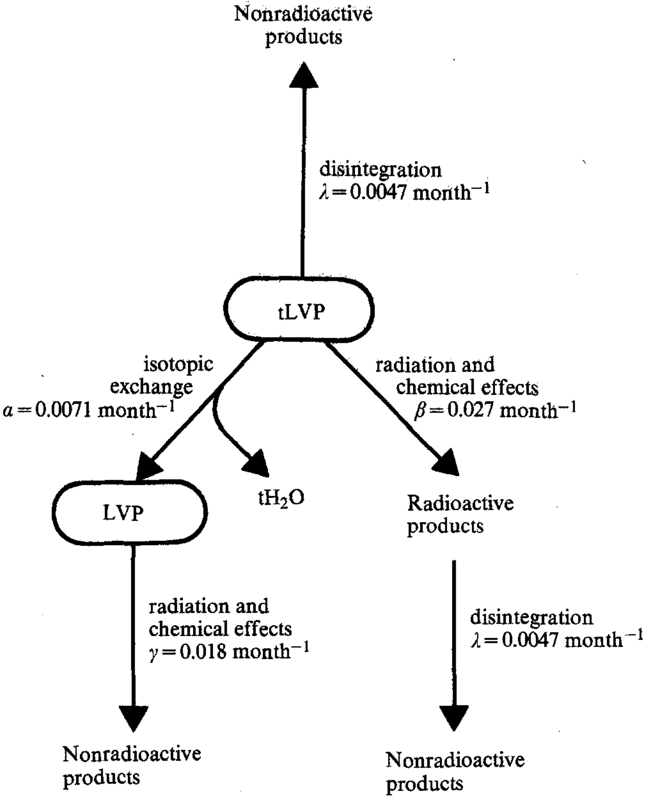

Fig. 2. Assumed decomposition of tLVP. tH_2O denotes tritiated water, α, β, γ, λ stand for rate constants (first order) of transformation processes (cf. text).

The decomposition of nonlabelled LVP was assumed to follow a simple exponential.

$$a = a_0 e^{-\gamma' t}. \tag{7}$$

Data in figure 1, b, were employed to estimate the constant γ', which has a value of 0.0042 month^{-1} and does not appear to be recognizably different for 4 °C and 20 °C, probably due to the small magnitude for constants at both temperatures and relatively large error of the assay.

The accuracy of numeric values for individual rate constants must not be overestimated due to the setup of the experiment, analytical procedures which might furnish slightly different results in various times etc. However, they show quite clearly the relative magnitude of single constants and, accordingly, the significance of the individual processes for the overall decomposition of tLVP and LVP. 3 conclusions can be drawn: 1. the 'chemical' decomposition in the absence of radioactivity (at 4 and 20 °C) is the slowest process ($\gamma' \ll \gamma$); 2. the isotopic exchange tLVP→LVP is also very slow; 3. the radiation damage to the tritiated substance seems to be somewhat larger than to the nonlabelled substance present in the same solution (cf. difference of rate constants γ and β). Compared in terms of half-lives, the chemical decomposition (processes 2 and 3) shows values of 29.4 and 55.5 months for labelled and nonlabelled substances, respectively, whereas in the nonlabelled preparation it reaches 238 months. An exposure to radioactivity apparently increases the rate of chemical changes considerably. The half-time of the isotopic exchange in our experiments was 141 months.

We would like to make 2 summarizing remarks on the stability of the investigated peptides. 1st, that the high stability of nonlabelled lysine vasopressin may perhaps relieve, at least partly, anxiety about large losses of active substance in pharmaceutical or laboratory preparations of LVP in the course of normal manipulation. 2nd, that our results for tLVP were obtained for a peptide possessing a specific radioactivity which is considered as rather low (2 Ci/mmole). It is impossible to foresee to what extent the same conclusions would be applicable for highly radioactive substances.

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