Isolation of Several Peptides with Relaxin Activity

Abstract. When a relaxin preparation was subjected to counter-current distribution for 355 transfers in a butanol–trichloroacetic acid system, significant biological activity was found over a band appreciably wider than was expected for a single component. By refractionation of the active band, three peptides with relaxin activity were isolated.

We have previously reported (*1*) that when aqueous ovarian extracts are distributed between 2-butanol and 1.0 percent trichloroacetic acid (TCA) the distribution of relaxin activity corresponds to that expected for a single molecular species, with $K = 2.7$ to 3.0. It was subsequently discovered that the system 2-butanol/0.2 percent TCA afforded results more favorable for purposes of purification, since the partition coefficient for relaxin in the latter system lay between 0.7 and 0.9, while the bulk of inactive material concentrated in the upper phase. More extensive experiments with relaxin in the 2-butanol/0.2 percent TCA system indicated the existence of more than one substance with relaxin activity. The isolation and partial purification of three active peptides is described in this report.

Relaxin was prepared from acetone-dried sow ovarian powder by the method of Frieden and Layman (*2*). Nine and seventy-five hundredths grams of a preparation containing 40 GPU/mg was distributed in separatory funnels between 2-butanol and 0.2 percent TCA; the volume of each phase was 250 ml. Eleven transfers were applied. The material in funnels 4 to 8, which included most of the activity and 17 percent of the total starting material (as measured spectrophotometrically at 285 mμ) was recovered and distributed between the same solvents in the Craig apparatus for 100 transfers. More than 80 percent of the original activity was located in tubes 36 to 60. This fraction was recovered and redistributed as before. After 105 transfers the contents of tubes 0 to 34 and 66 to 99 were removed and replaced by fresh solvents, and the distribution was continued for another 250 transfers with the apparatus arranged for recycling. The concentration of solute in each tube was measured spectrophotometrically, and relaxin activity was determined in pooled aliquots from 11 or 12 tubes over a band 67 tubes wide.

The results are shown in the upper half of Fig. 1. The solid line represents the solute concentration (left ordinate), and the shaded bars indicate relaxin concentration (right ordinate). The broken line is the distribution calculated for a substance with a distribution coefficient of 0.7, corresponding to the peak observed at tube No. 45. It may be observed that significant relaxin activity is found over a band appreciably wider than would be expected for a single substance.

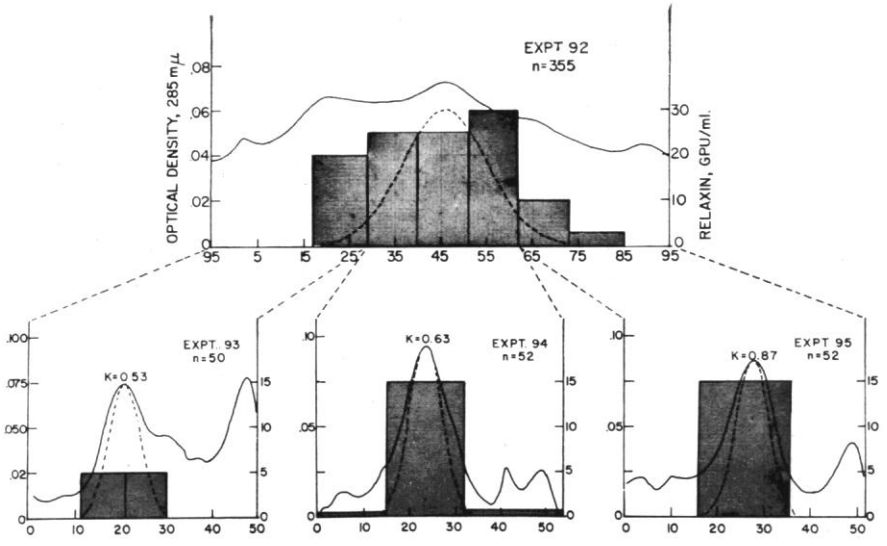

Fig. 1. (Top) Distribution of relaxin for 355 transfers in the system 2-butanol 0.2 percent TCA. (Bottom) Distribution of material pooled as indicated from experiment 92 for 50 to 52 transfers in the same system. Solid lines indicate optical density at 285 mμ (left ordinate); shaded bars indicate relaxin concentrations (right ordinate). The broken lines represent theoretical distribution curves. Abscissa: tube number.

The material from experiment No. 92 was pooled as indicated (tubes 29 to 61, 62 to 94, and 95 to 28); each fraction was concentrated, the excess TCA was removed by dialysis, and the recovered material was rerun in the same system for 50 to 52 transfers. Three peaks with relaxin activity were identified, with K's of 0.53, 0.63, and 0.87, respectively (lower half of Fig. 1). As before, relaxin concentrations are indicated by shaded bars, calculated distributions by broken lines. Peptide A ($K = 0.53$) was contaminated with a faster moving component; the configurations of peaks B and C ($K = 0.63$ and 0.87, respectively) were such as to indicate that each might contain up to 25 percent of material other than the main component. Quantitatively, the material recovered in the three active peaks totaled about 1 percent of the weight of the original preparation.

Paper chromatography of acid hydrolyzates of the three active peaks indicated the presence in each of the following amino acids: Asp, Ala, Arg, $(CyS)_2$, Glu, Gly, His, Lys, Ser, Thre, Val, and two leucines. From hydrolyzates of the DNP-derivatives of the intact-peptides, only ε-DNP lysine could be identified. All of the relaxin-active peptides which have been isolated exhibit an absorption spectrum characterized by a broad, nearly flat inflection in the region 255 to 285 mμ, ($E^{270}_{1\%} = 5.5$ to 7.5). The reason for this is unknown, since neither tyrosine nor phenylalanine could be detected in their acid hydrolyzates, and less than 0.5 percent tryptophan was found in the intact peptides by the method of Spies and Chambers (*3, 4*).

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References

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