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Discovery of novel albumin binding peptides using phage display and rational design

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FERRING PHARMACEUTICALS

ABSTRACT

Binding of peptides, lipids, and small molecules to human serum albumin (HSA) continues to be an attractive approach to extend the *in vivo* half-life of drug candidates. Since binding to HSA often affects the functional activity of these molecules, there remains a need for the discovery of unique HSA-binding motifs. Using phage display, we identified a small, cyclic peptide with micromolar affinity to albumin. During affinity maturation of this lead, it was found that the binding affinity could be improved by approximately ten-fold. The X-ray crystal structure of this peptide in complex with HSA suggested that there was little chance for further optimization due to the geometry of the binding pocket. A preliminary pharmacokinetic study in mice demonstrated that the half-life of this peptide after intravenous administration was 1.4 hours. Thus, this effort yielded novel HSA binding peptides that could serve as starting points for the development of new half-life extension motifs.

METHODOLOGY

Phage panning: Panning was done in four rounds with human serum albumin (Sigma) coated on plates. Phage libraries were based on a pentavalent vector with alpha-helical, beta-turn, linear, and disulfide-bonded cyclic motifs of various amino acid lengths.

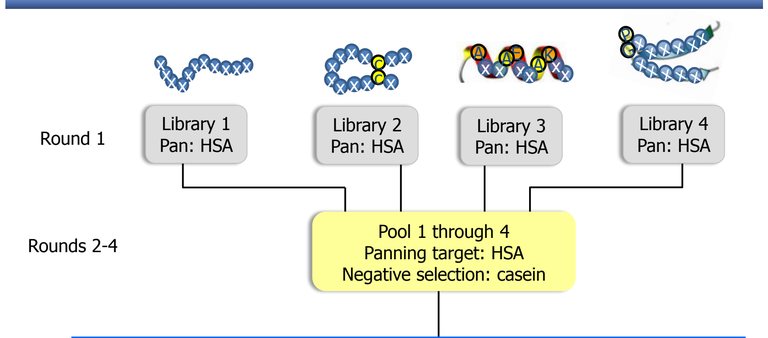
Focused library optimization: Focused phage panning was done with HSA, similar to the primary panning. Focused phage libraries were generated with stretches of five randomized positions covering the length of the peptide sequence, excluding the cysteine residues.

SPR analysis: Phage hits were synthesized and tested with Biacore 3000 with CM5 chips for binding affinity measurements to human and rat serum albumin. Analyses were done based on one or two runs.

Crystal structure: Data were collected at Shanghai synchrotron. Three data sets were collected and merged with diffraction of 2.0 Å. Structure was determined with molecular replacement using HSA as a model.

Rat PK study was performed with IV bolus administration of four male Sprague Dawley rats using 5% mannitol in water as vehicle.

PANNING STRATEGY



1. Ser-Leu-Tyr-Cys-Tyr-Met-Tyr-Asp-Tyr-Lys-Gly-Glu-His-Cys-Ile-Asp-Asn
2. Leu-Ala-Pro-Cys-Tyr-Phe-Arg-Val-Ala-Glu-Ile-Glu-Val-Cys-Trp-Asp-Pro
3. His-Phe-Ser-Asn-Ser-Asp-Phe-Tyr-Tyr-Met-Phe
4. Val-Leu-Asp-Trp-Tyr-Thr-Trp-Asn-Glu-Leu-Trp

RESULTS

AFFINITY DETERMINATION BY SPR ANALYSIS

ID	Sequence	Biacore steady-state affinity (μM) Human albumin	Rat albumin
N491781	Ac-Ser-Leu-Tyr-c(Cys-Tyr-Met-Tyr-Asp-Tyr-Lys-Gly-Glu-His-Cys)-Ile-Asp-Asn-NH2	4.6	145
N514493	Ac-Leu-Ala-Pro-c(Cys-Tyr-Phe-Arg-Val-Ala-Glu-Ile-Glu-Val-Cys)-Trp-Asp-Pro-NH2	1.7	2.6
N284723	Ac-Val-Leu-Asp-Trp-Tyr-Thr-Trp-Asn-Glu-Leu-Trp-Lys-Lys-Lys-NH2	13.4	17.4
N19347	Ac-His-Phe-Ser-Asn-Ser-Asp-Phe-Tyr-Tyr-Met-Phe-NH2	N/A	N/A

- N/A = analysis performed but result inconclusive
- Lys-Lys-Lys added to increase solubility

AFFINITY MATURATION WITH PHAGE DISPLAY

N491781 maturation

ID	Sequence	Biacore steady-state affinity (μM) Human albumin	Rat albumin
N777751	Ac-Ser-Leu-Tyr-c(Cys-Tyr-Nle-Tyr-Asp-Tyr-Lys-Gly-Glu-His-Cys)-Ile-Asp-Asn-NH2	37.5	312
N664815	Ac-Ser-Leu-Tyr-c(Cys-Tyr-Nle-Tyr-Asp-Tyr-Arg-Gly-Asn-Arg-Cys)-Ile-Asp-Asn-NH2	13.6	180
N607534	Ac-Ser-Leu-Tyr-c(Cys-Tyr-Nle-Tyr-Asp-Tyr-Arg-Gly-Asp-Pro-Cys)-Ile-Asp-Asn-NH2	3	17.2
N576826	Ac-Ser-Leu-Tyr-c(Cys-Tyr-Nle-Tyr-Asp-Tyr-Lys-Gly-Asp-Arg-Cys)-Pro-Asn-Asn-NH2	3.7	11.2
N706671	Ac-Ser-Leu-Tyr-c(Cys-Tyr-Nle-Tyr-Asp-Tyr-Lys-Gly-Asp-Arg-Cys)-Arg-Glu-Asp-NH2	4.5	14.8
N482080	Ac-Ser-Leu-Tyr-c(Cys-Tyr-Nle-Tyr-Asp-Tyr-Lys-Gly-Glu-Arg-Cys)-Arg-Lys-Glu-NH2	2.7	11.6
N135734	Ac-Ser-Leu-Tyr-c(Cys-Tyr-Nle-Tyr-Asp-Tyr-Lys-Gly-Asp-Pro-Cys)-Arg-Arg-Lys-NH2	N/A	N/A

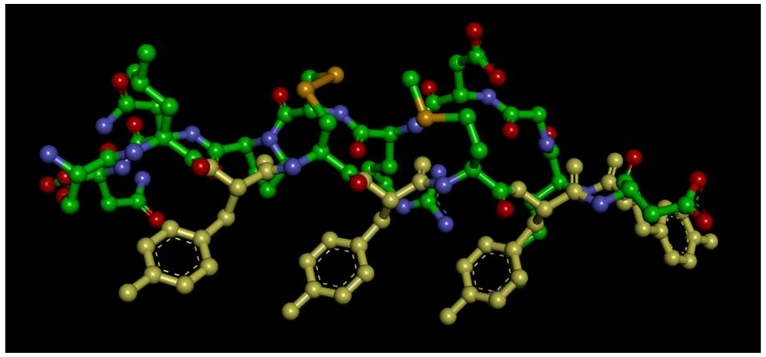
- N/A = analysis performed but result inconclusive
- Purple = Randomized positions
- Nle used to replace Met for synthesis purpose
  - Phage display was not done with Nle
  - N777751 = N491781 with Nle in place of Met
  - Nle reduced affinity by ~10-fold (human) or ~2-fold (rat) **(A)**
- Maturation improved affinity to human and rat albumin **(B)**

Nle replacement and additional maturation

ID	Sequence	Biacore steady-state affinity (μM) Human albumin	Rat albumin
N425069	Ac-Ser-Leu-Tyr-c(Cys-Tyr-Met-Tyr-Asp-Tyr-Lys-Gly-Asp-Arg-Cys)-Pro-Asn-Asn-NH2	0.42	2.0
	Combination of libraries, modeling & design:		
N564606	Ac-Tyr-Met-Tyr-c(Cys-Tyr-Arg-Tyr-Asp-Tyr-Lys-Gly-Asp-Arg-Cys)-Arg-Asn-Asn-NH2	0.6	11
N253411	Ac-Tyr-Met-Tyr-c(Cys-Tyr-Met-Tyr-Asp-Tyr-Lys-Gly-Asp-Arg-Cys)-Pro-Asn-Asn-NH2	0.7	2.5
N000476	Ac-Tyr-Met-Tyr-c(Cys-Tyr-Met-Tyr-Asp-Tyr-Arg-Gly-Glu-Arg-Cys)-Arg-Lys-Glu-NH2	0.9	6.2

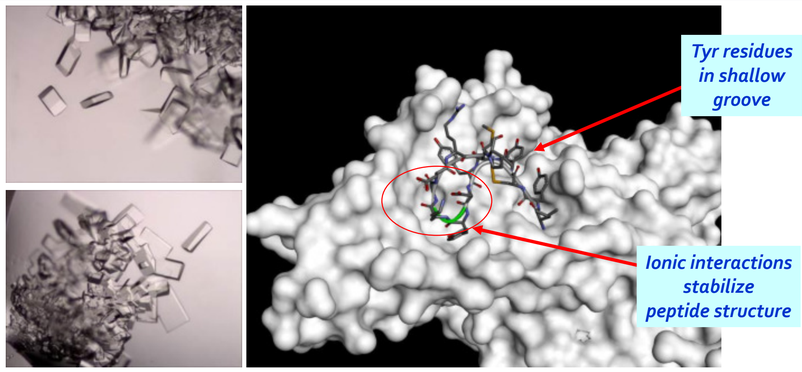
- Nle replaced back to Met
- Affinity improvement from parental N491781 **(C)**
  - ~10-fold to human albumin
  - ~50-fold to rat albumin
- Additional mutations did not increase affinity

MODELING OF TYROSINE RESIDUES



(A)

CO-CYSTAL STRUCTURE WITH ALBUMIN



(B)

(C)

PRELIMINARY RAT PK ANALYSIS

ID	Rat albumin affinity (μM)	Rat CL_{iv} ml/min/kg	Rat $t_{½}$ IV bol, hours
N425069	2.0	0.8	1.4

CONCLUSIONS

Binding to albumin continues to be an attractive approach for half-life extension. We used a phage panning strategy to discover novel human albumin-binding peptides. One peptide was selected for affinity maturation, and the Kd to human and rat albumin was improved by 10- and 50-fold, respectively. However, further optimization by rational design failed to produce a higher affinity peptide. Peptide modeling and X-ray crystallography provided some clues to the limit of optimization for this peptide. Preliminary rat PK analysis did show that the final optimized peptide has a half-life of 1.4 hrs with IV bolus administration, a ten-fold improvement over that of typical peptides in rats. This effort validates the phage panning approach for the discovery of novel albumin-binding peptides.